Collagen Chemoembolization: Pharmacokinetics and Tissue Tolerance of cis-Diaminedichloroplatinum(II) in Porcine Liver and Rabbit Kidney

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ABSTRACT

Pharmacokinetics of chemoembolization with a fibrous collagen carrier was studied in rabbit kidney and porcine liver models. Cisplatin (1 mg/ml) chemoembolization of liver and kidney was compared with i.v. and intraarterial cisplatin infusion. Tissue platinum concentration [Pt] was measured at 2.5 h by atomic absorption spectrometry. Renal platinum retention and Angiostat (collagen for embolization) concentration were linearly related (r = 0.87, p < 0.001). At 10 mg/ml collagen for embolization, chemoembolized kidney [Pt] was 220 ± 50 (SE; n = 4) times contralateral kidney [Pt], and 62 and 23 times renal [Pt] by i.v. and intraarterial infusion, respectively. At 10 mg/ml collagen for embolization, chemoembolized liver [Pt] was 2 times hepatic [Pt] by i.v. and intraarterial infusion. Since hepatic tumor vasculature is end arterial, chemoembolization should yield high [Pt] in tumor (as in kidney) but lower levels in normal liver.

INTRODUCTION

Chemoembolization refers to the intraarterial coadministration of chemotherapeutic and vascular occlusive agents to treat malignant disease. Vascular occlusion prolongs dwell time of the antineoplastic agent within the tumor, increasing first pass fractional extraction of drug. Additional therapeutic effect may be obtained by tumor ischemia. In liver, chemoembolization offers a special opportunity for selective drug delivery. The blood supply of tumor in liver is principally arterial, while hepatocytes are also bathed by portal blood flow (1). The portal blood flow supports hepatocytes during ischemia and clears drug from hepatocytes but not from areas of malignant disease in liver.

Chemoembolization has been studied in several organ sites, using a variety of embolic materials and drugs (2-7). The majority of clinical hepatic chemoembolization experiences have used delivery of drug within biodegradable microspheres and microcapsules (8-13). Other embolic materials which have been explored include coaxial balloon catheters (14), Gel foam, Ivalon, and stainless steel coils (15). To avoid tissue infarction, embolic materials are generally either large enough to occlude proximal to preexisting collateral vessels or are labile and produce only transient occlusion.

We have developed a collagen particle [Angiostat (CFE3)] specifically to address the requirements for efficient chemoembolization of malignant disease. Design considerations included degree of dwell time prolongation, distal penetration within the vascular bed, interaction between drug and particle, degree of ischemic injury, and reversibility of vascular occlusion.

MATERIALS AND METHODS

Reagents. HPLC grade organic solvents were obtained from J. T. Baker Chemical Co. All other chemical reagents and standards were purchased from Sigma Chemical Co. unless otherwise specified.

Analytic Methods. Platinum levels in tissue homogenates, fluids, and in ultratrifilates of fluids were determined by flameless AAS, utilizing a Perkin-Elmer Model 2380 atomic absorption spectrophotometer and a heated graphite atomizer with an HGA-400 programmer. Sample aliquots of 20 µl were introduced and carried through a four-stage furnace heating program. Liquid phase evaporation was achieved by temperature ramp from ambient to 90°C over 45 s and held for another 60 s. Complex sample matrices were then volatilized in a charring step by ramping the furnace temperature to 1450°C over 40 s and holding for 30 s. During this 70-s interval, automatic base-line correction occurred at 62 s, the argon gas flow (300 ml/min) was interrupted at 65 s, and a spectrophotometer-read cycle was initiated at 69 s, i.e., 8, 5, and 1 s prior to atomization, respectively. The atomization step used "max power" (zero ramp time), heating to 2650°C which was maintained for 5 s under gas flow arrest. Integrated absorbance (peak area) was obtained during atomization, and sample platinum concentration was determined from a machine calibration curve. A final 6-s 2650°C burnoff under resumed gas flow conditions was added to reduce residual analyte between samples.

Aqueous platinum standards were prepared from a standard solution (1000 µg/ml in 0.01 N HCl) by serial dilution with 0.2% HNO3. Weighed tissue samples were homogenized in a glass homogenizer with a known volume of 0.25% Triton X (New England Nuclear) and aliquots were taken for triplicate AAS determinations. Tissue concentrations are expressed as µg platinum per g wet weight of tissue.

Reactive cisplatin (Bristol Laboratories) in fluids was estimated by measuring the DDTC derivative, using HPLC. The sample extraction procedure was adapted from Andrews et al. (17). To 0.5-ml sample aliquots were added 5 µl of 0.55 mg/ml NiCl2, internal standard, and 50 µl freshly prepared 10% DDTC in 0.1 N NaOH (w/v). The mixture was incubated for 30 min at 37°C, then chilled on ice. The platinum and nickel DDTC derivatives were extracted with 0.2 ml CHCl3 by thorough vortex mixing for 1 min. Separation into aqueous and organic layers was obtained by centrifugation at 1000 × g for 10 min. The
chloretone (bottom) layer was filtered (0.22 μm-filter) and a 20-μl aliquot was injected into the HPLC.

Aqueous samples were chromatographed on a Waters HPLC system consisting of 2 Model 501 solvent delivery pumps, a Model U6K injector, Model 680 automated gradient controller, Model 441 absorbance detector, Model 740 data module, and a 3.9-mm × 30-cm C18-μBondapak column. A 4:1 methanolwater(v/v) mobile phase was delivered at 1 ml/min, and column effluent was monitored at 254 nm with a sensitivity of 0.01 absorbance unit full scale. Retention times for platinum and nickel DDTC complexes were 7.0 and 8.0 min, respectively. Sample concentrations were determined from a standard curve of the ratio of peak areas for the Pt(DDTC)2 and Ni(DDTC)2 peaks. The standard curve was linear (r = 0.999) over the range of 1 to 10 μg/ml CDDP.

Drug Dialysis. To measure potential binding between cisplatin and Angiostat, CFE (Target Therapeutics), the rate of drug efflux from a dialysis bag containing either drug alone or drug and collagen was determined. Drug and drug-CFE mixtures were prepared in diatrizoate meglumine 66% and diatrizoate sodium 10% (Hypaque-76, Winthrop-Breun Laboratories). The iodinated contrast media were included to model conditions of in vivo administration. Dialysis tubing (Union Carbide; putative pore size, 12,000 to 14,000 daltons) was prepared by boiling in multiple changes of water with EDTA. Dialysis bags contained 1 ml of the mixture to be studied. Final concentrations were 10 mg/ml CFE, 1 mg/ml CDDP, and 54% diatrizoate. Control bags were prepared without collagen. Dialysis with stirring was with 200 ml phosphate-buffered saline, pH 7.4, at 37°C. Dialysate was sampled at 0, 10, 30, 60, 120, 180, and 240 min. CDDP levels were determined by HPLC.

This design approximates the transfer of a solute within a closed two-compartment system such that equilibrium is approached at a single exponential rate. Half-times were determined from the slope of the natural log of the difference between the concentration-time data and the expected equilibrium concentrations, namely, the total amount of drug in the system divided by the system volume. Because some measurements exceeded the expected equilibrium value, slightly higher asymptotes were chosen for calculations.

Renal Chemoembolization. Rabbit kidney was used to model chemoembolization in an end-artery system and to measure the effect of collagen concentration upon tissue chemotherapy retention. Tissue platinum deposition was compared following administration of 0.4 mg CDDP by i.v. infusion (3 rabbits), left renal i.a. infusion (4 rabbits), and left renal chemoembolization with variable concentrations of CFE (24 rabbits). Male New Zealand White rabbits weighing 4 to 5 kg were preanesthetized with intramuscular injections of 30 mg/kg sodium pentobarbital (Nembutal; Abbott Laboratories) and 30 mg/kg ketamine HC1 and 0.4 mg/kg xylazine (Rompun; Miles Laboratories) administered i.m. Anesthesia was maintained with i.v. sodium pentobarbital in a running normal saline line. The animals in the i.v. treatment group received 0.4 ml of 1 mg/ml CDDP in 76% iodinated contrast infused into the ear vein. In the remaining animals, the right femoral artery was dissected free and a distal ligature was placed. An arteriotomy was performed and a graded stiffness infusion catheter and 0.016-inch Taper steerable guide wire (Target Therapeutics) were introduced. At this point, 4000 units heparin (Invenex Laboratories) and 250 mg ampicillin sodium (Wyeth Laboratories) were administered by i.a. injection. The catheter was placed within a segmental hepatic artery under fluoroscopic guidance. Chemoembolic mixtures were prepared by solubilizing CDDP in 76% iodinated contrast at 2 × final concentration and mixing 1:1 (v/v) with CFE at 20 mg/ml by exchange between syringes, using a 3-way stopcock. The chemoembolic mixture was administered until retrograde flow was observed. The catheter was then removed, a proximal ligature was placed for hemostasis, and the wound site was closed with 3-0 silk. After chemoembolization, antibiotic coverage consisted of 1300 units/kg/day penicillin G procaine (E. R. Squibb & Sons) by i.m. injection for 3 days. Blood samples were drawn for hematological and serum chemistry evaluations at base line and at 3, 7, 14, and 21 days following chemoembolization. Animals were sacrificed on day 21 for gross examination and for histological evaluation of liver, gallbladder, duodenum, spleen, pancreas, and kidney. Percentage of liver damage was estimated by visualization of gross specimens and, wherever possible, by weight. Two additional animals underwent hepatic chemoembolization with 1 mg/ml CDDP and 10 mg/ml CFE with repeat chemoembolization at 2 weeks. These animals were sacrificed 5 weeks following initial chemoembolization, and cholangiography was performed to evaluate patency of the biliary tree.

Plasma clearance of total platinum and acute (2.5 h) tissue deposition were determined in nine Duroc pigs weighing 23 to 27 kg. Platinum levels were determined following administration of 0.4 mg/kg (1 mg/ml) CDDP in three animals each by i.v. infusion, hepatic artery infusion, and hepatic chemoembolization with 4 mg/kg (10 mg/ml) CFE. All drug mixtures were prepared in 66% diatrizoate meglumine and 10% diatrizoate sodium and were delivered at approximately 2 ml/min. Blood samples were obtained from a venous line immediately following drug delivery and at 10, 20, 30, 40, 50, 60, 90, 120, and 150 min thereafter. Serum was separated immediately at 1000 x g and removed for AAS measurement of total platinum. Animals were sacrificed at 2.5 h, and liver, spleen, pancreas, and both kidneys were removed, weighed, and prepared for [Pt] determination.

RESULTS

In Vitro Drug Binding. Apparent drug binding by CFE was limited. The concentration-time curve for appearance of drug into dialysate was slightly retarded in the presence of CFE in the dialysis bag. The half-times for CDDP alone and for drug plus CFE were 35 and 64 min, respectively. At all time points examined, the presence of CFE affected dialysate drug concentration by no more than 21%, with an average difference of 11%.

Renal Chemoembolization. Chemoembolization with CFE and cisplatin led to substantial increases in drug retention by the target kidney. Renal and hepatic mean platinum levels following i.v. CDDP infusion, left renal i.a. infusion, and chemoembolization are shown in Table 1. Systemic CDDP administration produced an equivalent distribution between left and right kidneys with 0.5% of the administered dose recovered in each kidney at 2 h. Following i.a. infusion, the target left kidney retained 1.4% of the administered dose with a mean platinum level 5.4 times that of contralateral kidney and 3 times the concentration after i.v. administration. In chemoembolization groups, increased target tissue drug retention and decreased delivery to nontarget areas was a function of CFE concentration (Fig. 1). Regression analysis and calculation of the F statistic indicate a direct linear relationship (r = 0.87; P < 0.001) between target tissue platinum and collagen concentration. At 10 mg/ml CFE, the chemoembolized kidney retained 38% of the administered dose, 220 times the concentration in the contralateral kidney, and 62 and 23 times renal levels obtained following i.v. and i.a. infusion, respectively. Similar results were
within right kidney and liver, is a function of CFE concentration. The efficiency with which cisplatin is retained within the left kidney and reduced with 0.4 ml of 1 mg/ml cisplatin and variable collagen (CFE) in the rabbit. A rapid early serum half-time following i.v. infusion, probably reflecting inadvertent chem embolization were slightly higher than those observed following hepatic chemoembolization. Consistent with the reduction of tissue platinum in noninfused organs. Compared with i.v. administration, chemoembolization resulted in a 10 to 30% reduction of tissue platinum in noninfused organs. Splenic and pancreatic tissue levels following hepatic chemoembolization were significantly higher than those observed following i.v. infusion, probably reflecting inadvertent chemoembolization of those organs. A rapid early serum half-time of 17 min was observed for both i.v. and i.a. groups and not seen following chemoembolization.

Table 2 Cisplatin pharmacokinetics: effect of i.v. infusion, hepatic i.a. infusion, and hepatic chemoembolization (mean ± SE)

<table>
<thead>
<tr>
<th>Route</th>
<th>N</th>
<th>Peak Pt (µg/ml)</th>
<th>AUC (µg/ml/min)</th>
<th>Liver Tissue Pt deposition (µg Pt/g wet wt)</th>
<th>Kidney Tissue Pt deposition (µg Pt/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion i.v.</td>
<td>3</td>
<td>3.53 ± 2.38</td>
<td>88.6 ± 37.6</td>
<td>0.457 ± 0.092</td>
<td>0.660 ± 0.106</td>
</tr>
<tr>
<td>Infusion i.a.</td>
<td>3</td>
<td>1.09 ± 0.24</td>
<td>46.4 ± 4.5</td>
<td>0.457 ± 0.081</td>
<td>0.859 ± 0.027</td>
</tr>
<tr>
<td>Chemoembolization</td>
<td>3</td>
<td>0.40 ± 0.03</td>
<td>37.6 ± 1.3</td>
<td>0.907 ± 0.296</td>
<td>0.437 ± 0.034</td>
</tr>
</tbody>
</table>

* AUC, area under the plasma clearance curve.

obtained by using ionic and non-ionic contrast media.

**Hepatic Chemoembolization.** Drug distribution following porcine hepatic chemoembolization with cisplatin is presented in Table 2 and in Figs. 2 and 3. No difference in normal hepatic tissue platinum levels was observed between i.v. and hepatic i.a. infusion groups, whereas chemoembolization resulted in a 2-fold increase in hepatic platinum deposition. At 10 mg/ml collagen, hepatic retention of platinum at 2.5 h was 4% of the administered dose for i.v. and i.a. infusions, and 7% following chemoembolization. The mean coefficient of variance between specimens obtained from the same liver was about 10% for i.v. and i.a. infused groups and 30% for chemoembolized liver. Compared with i.v. administration, chemoembolization resulted in a 10 to 30% reduction of tissue platinum in noninfused organs. Splenic and pancreatic tissue levels following hepatic chemoembolization were slightly higher than those observed following i.v. infusion, probably reflecting inadvertent chemoembolization of those organs. A rapid early serum half-time of 17 min was observed for both i.v. and i.a. groups and not seen following chemoembolization. Consistent with the reduction of platinum deposition in nontarget organs was a reduction by 60% of the area under the plasma clearance curve for total platinum following chemoembolization as compared with i.v. delivery. Late phase clearance was similar for all groups.

Fig. 2. Porcine tissue platinum deposition 2.5 h following administration of 0.4 mg/kg (1 mg/ml) CDDP by i.v. infusion, hepatic i.a. infusion, and hepatic chemoembolization (CE) with 10 mg/ml CFE. Relative to i.v. infusion, hepatic deposition was unchanged by i.a. infusion, and doubled following chemoembolization. Modest changes in nontarget tissue levels were also observed.

Fig. 3. Porcine plasma platinum clearance curves following cisplatin by i.v. infusion (Δ), hepatic i.a. infusion (○), and hepatic chemoembolization (▲). Peak levels were substantially reduced by hepatic regional delivery. Late phase clearance was similar for all groups.

Dose-effect relationships for liver toxicity were examined in the porcine liver model for various concentrations of cisplatin at 10 mg/ml CFE. When present, liver damage at day 21 was characterized by necrosis which ranged from focal to massive, intrahepatic fibrous tissue, and adhesions to adjacent structures. Lesions were often encapsulated with a friable core. Liver damage was scored on the basis of percentage of liver with macroscopic damage. Increasing concentrations of CDDP resulted in a higher frequency and larger area of tissue damage (Fig. 4).

Transient liver dysfunction measured by serum enzyme levels was generally resolved by 1 to 3 weeks. Minimal elevations in WBC and decreases in platelets were resolved by day 21.
severity of serum enzyme abnormalities correlated well with the degree of liver damage present at necropsy.

Hepatic chemoembolization was generally tolerated without significant liver damage at up to 7 mg/ml CDDP.

Repeat chemoembolization 2 weeks following initial hepatic administration of CFE:CDDP 10:1 mg/ml resulted in minimal liver necrosis in two animals. In one case, transient 5- and 10-fold elevations in total bilirubin were observed 1 week following initial and repeat procedures, respectively. A postmortem cholangiogram showed stenotic areas to be present throughout the biliary tree.

Three animals were not included in the results due to inadvertent chemoembolization of the gastroduodenal artery which became apparent at necropsy. These animals survived or were sacrificed less than 1 week following chemoembolization. Severe gastroduodenal infarction was present in all cases.

DISCUSSION

Chemoembolization provides both enhanced confinement of drug within the tumor bed and direct ischemia due to vascular occlusion. The relative advantage of regional drug delivery is a function of first pass extraction and of systemic half-life of the drug (18). For several chemotherapeutics, only limited therapeutic advantage is gained by intraarterial as compared to systemic administration (18–20). Chemoembolization increases first pass extraction by increasing drug dwell time. As regional delivery becomes more efficient, however, local toxicity may become the dose limiting factor. Thus, in hepatic artery chemoembolization the relative distribution of drug between normal hepatocytes and cancer in liver may determine the therapeutic index and ultimate effectiveness. After hepatic artery chemoembolization, we hypothesize that portal blood flow will selectively reduce retention of drug by normal liver as compared with malignant disease in liver where the portal system has been displaced.

In vitro studies indicate that CDDP is not significantly bound by CFE, making it suitable as a passive carrier for this agent. CFE (10 mg/ml) may be safely delivered with up to 7 mg/ml CDDP as indicated by hepatic tolerance studies. It is suggested that tolerance to hepatic chemoembolization is improved with antibiotic protection. 4 In general, transient elevation of liver function tests was resolved by 3 weeks. Higher drug concentrations resulted in liver necrosis.

In the present study we have measured platinum deposition in normal liver and have used normal kidney to model the end-artery kinetics of drug delivery to cancer in liver. Administration of cisplatin by i.v. and hepatic artery i.a. resulted in similar hepatic platinum levels of 0.46 μg/g tissue at 2.5 h. After chemoembolization, hepatic levels were increased 2-fold to 0.91 μg/g. Renal artery chemoembolization, however, resulted in much higher 2.5-h renal platinum levels of 10.1 μg/g, resulting in an apparent 11:1 ratio for drug delivery by chemoembolization in end artery as compared with circulations influenced by portal flow. In a concurrent clinical trial, CDDP concentrations of 10 mg/ml have been achieved. Extrapolating from the model data presented here, vascularized tumor tissue should achieve platinum levels of 100 μg/g wet weight compared with 0.2 to 0.6 μg/g, the range noted in several tissues following i.v. administration, a 200-fold increase in cisplatin delivered to tumor.

The general validity of these estimates is supported by determination of Pt distribution in patient livers containing primary and metastatic cancer. Livers from five patients were obtained at necropsy or upon surgical resection. Chemoembolization with 10 mg/ml collagen and 1 mg/ml cisplatin had been performed prior to death or surgery. Liver computer-assisted tomography scans at completion of embolization demonstrated contrast arrest within the periphery of the tumor. Platinum deposition generally was highest at the tumor rim, successively lower in tumor core and normal liver, and lowest in extrahepatic tissues.

Limited hepatic vascular capacitance coupled with increased first pass extraction of drug results in relatively low systemic exposure at drug concentrations within the range of hepatic tolerance. We documented decreased levels of platinum in serum and nontarget organs following hepatic chemoembolization. In initial clinical trials patients have received an average volume of 8 ± 7 ml at concentrations as high as CFE:CDDP 10:10 mg/ml. 4 We calculate that the systemic exposure required to achieve these high tumor concentrations of drug is approximately 50% of a typical systemic dose.

Chemoembolization with other agents, namely mitomycin C, doxorubicin, fluorodeoxyuridine, 1,3-bis(2-chloro)-1-nitrosourea, and etoposide, and with drug combinations is being studied in a similar manner. 4 The effect of antibiotic protection on hepatic tolerance to chemoembolization are currently being studied in both animal models and concurrent clinical trials. It is unclear at present what effects vascular occlusive changes in physiological microenvironment such as hypoxia, reduced pH, and energy status have on drug uptake and cytotoxicity. These considerations warrant additional future study.

The present study documents a differential relative advantage in drug deposition in end-artery systems over that observed for normal liver. The establishment of safe concentration ranges for hepatic chemoembolization has made possible the initiation of Phase I clinical trials. Data from these trials are being assembled and will be reported separately.

ACKNOWLEDGMENTS

The authors would like to thank Tim and Sue Moore of Irish Farms (Norco, CA) who supplied and cared for the animals in this study.

4 Unpublished observations.

5 Assuming a typical cisplatin i.v. dose of 2 mg/kg in a 70-kg recipient results in a 140-mg total body exposure. The maximum chemoembolic dose will be (8 ml) x (10 mg/ml) or 80 mg. Seven % regional entrapped further reduces this to a 74-mg dose equivalent or 53%.
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