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A phase I and pharmacokinetic comparison of hepatic arterial and peripheral vein infusions of bisantrene for liver cancer*

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Summary. Bisantrene (NSC-337766) was administered to five patients with cancer of the liver (one case of hepatocellular carcinoma, two of metastatic carcinoma of unknown primary, two of metastatic colorectal carcinoma). Under fluoroscopic guidance, percutaneous hepatic venous catheters were placed in five patients and percutaneous hepatic arterial catheters in four. A fifth patient's hepatic arterial catheter was implanted at laparotomy. Hepatic plasma flow was estimated by the Fick principle using peripheral vein indocyanine green infusion. On the first day of treatment, patients received a 2- or 4 h hepatic arterial infusion of bisantrene (130 mg/m^2); peripheral venous, hepatic arterial, and hepatic venous timed blood samples were drawn during and for 18 h after drug infusion. On the second day of treatment, 2- or 4 h peripheral vein infusion of bisantrene (130 mg/m^2) was followed by the same blood sampling schedule. Patients were followed weekly for toxicity. Four patients received only one course of treatment, while a fifth received two courses. All patients experienced leukopenia (median nadir 2400/mm^3; range 1400–2700/mm^3). Two patients developed fever after drug infusion. No antitumor responses were observed. Plasma bisantrene concentrations were measured by HPLC. Pharmacokinetic analyses are reported for four patients. The hepatic extraction ratio ranged from 15% to 49%, hepatic plasma clearances were 0.029–0.353 l/min/m^2; peripheral vein areas under the concentration–time curve during hepatic arterial infusion ranged from 35% to 50% of peripheral vein areas under the curve during peripheral vein infusion. We conclude that hepatic arterial bisantrene infusion offers only modest pharmacokinetic advantage to the target organ or to the systemic circulation over peripheral vein infusion.

Introduction

Primary and metastatic cancers of the liver remain an important cause of morbidity and mortality for afflicted patients. Despite published experience with hepatic arterial infusion of antineoplastic agents exceeding 20 years, this technique has only recently become popular, with the development of improved technology allowing prolonged infusions. Several workers have administered 5-fluorodeoxyuridine via the hepatic artery with the Infusaid pump and have reported excellent response rates and extension of survival in patients with metastatic colorectal cancer [2, 12]. 5-Fluorodeoxyuridine is an agent with high hepatic extraction (69%–92%) [5] and offers the opportunity for delivering high concentrations of drug to the liver with little resultant systemic toxicity. As noted by Ensminger, Garnick, and coworkers, optimal therapeutic advantage for anticancer agents administered via the hepatic artery may be expected with those drugs found to have a high hepatic extraction ratio (HER) [5, 7]. In an attempt to identify agents with a pharmacokinetic basis warranting hepatic arterial infusion, this study sought to define the HER, hepatic plasma clearance, and peripheral vein drug concentrations for bisantrene during sequential hepatic arterial and peripheral venous infusions.

Bisantrene (9,10-anthracenedicarboxaldehyde bis [[4,5-dihydro-1*H*-imidiazol-2-yl]hydrazone] dihydrochloride; NSC-337766) is a derivative of the anthracene class of anticancer agents. The agent appears to have DNA-intercalating, strand-breaking, and cross-linking properties, although the manner in which cytotoxicity is induced is unknown [3, 10]. The agent is undergoing phase II and phase III clinical trials in the United States of America and Europe [1, 6, 11, 13, 15].

The pharmacokinetic behavior of bisantrene when administered by IV injection has recently been characterized [8, 14]. Both triexponential and biexponential plasma decay curves have been described. According to these studies, the terminal half-lives are 19 or 26 h, steady-state volumes of distribution vary from 627 l/m^2 to 1847 l/m^2, and mean plasma clearances are 710 and 735 ml/min/m^2. These values suggest substantial sequestration of bisantrene in tissues. In phase I trials, the maximum tolerated dose with single-IV-injection schedules was 260–280 mg/m^2 [1, 15]. For the purpose of this study, one half the maximum tolerated dose was administered on each of 2 consecutive days, the first dose by hepatic arterial infusion, the second by peripheral vein infusion.

Materials and methods

General requirements. Patients with histologically proven metastatic or primary cancer of the liver were eligible for

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this study. Patients' malignancies were refractory to all other forms of therapy. Patients had a life expectancy of 4 weeks, a Southwest Oncology Group performance status of 3 or better, and full recovery from toxicities of prior therapy. Patients underwent physical examination and laboratory studies to assure organ function compatible with adequate drug tolerance, metabolism and excretion. Signed informed consent was obtained from all patients.

All patients were hospitalized in a General Clinical Research Center. All five patients underwent transfemoral celiac and superior mesenteric arteriography. At the time of arteriography, the right hepatic artery was catheterized in two patients and the common hepatic artery was catheterized in another two patients. A fifth patient underwent surgical implantation of a silastic catheter in the right hepatic artery following ligation of the left hepatic artery. All patients' hepatic veins were cannulated under fluoroscopy. Patency of catheters was maintained with 5% dextrose in water containing 10 units/ml preservative-free heparin. Solutions were delivered by pump at 21 ml/h through three-way stopcocks.

Estimation of hepatic blood flow. Following a 10-mg IV loading dose of indocyanine green (ICG; Cardiogreen, Becton-Dickinson, Baltimore, Md), patients received an IV infusion of ICG at a rate of 0.3 mg/m^2/min. Peripheral venous, hepatic arterial, and hepatic venous blood samples were collected simultaneously every 15 min for 1 h. Serum was separated, frozen, and stored until analysis. Serum ICG concentrations were determined by spectrophotometric absorption at a wavelength of 805 nm and compared with a standard curve for dye concentration vs optical density. Hepatic plasma flow was estimated with reference to the Fick principle [9].

Drug infusion protocol. Following arteriography, catheter placement, and hepatic blood flow estimation, eligible patients received bisantrene on 2 separate days of infusion. Bisantrene was provided by the National Cancer Institute, Bethesda Md, as a sterile lyophilized powder in vials containing 50 mg or 250 mg drug as free base. Hepatic arterial infusion of one half the maximum tolerated dosage (0.5×260 mg/m^2 = 130 mg/m^2) was accomplished over 2 or 4 h on day 1, and peripheral vein infusion by an identical administration schedule on day 2. Bisantrene was prepared in 500 ml 5% dextrose in water. At 30 min before the administration of chemotherapy, patients received IV injections of 50 mg diphenhydramine and 100 mg hydrocortisone succinate. During and after each drug infusion, peripheral vein, hepatic vein, and (except during hepatic arterial infusion) hepatic artery blood specimens (3 ml) were collected in heparinized tubes. Specimens were drawn prior to infusion, at 30, 60, 90, 120, 180, and 240 min during infusion, and 15, 30, 60 and 90 min and 2, 4, 8, 12, and 18 h after infusion. Specimens were spun at 2000 *g* for 10 min; plasma was then decanted and frozen at −27 °C until high-performance liquid chromatography (HPLC) was performed.

All transfemoral catheters remained in place during blood sampling and were removed 24 h after the beginning of the final drug infusion (approximately 72 h after arteriographic placement). Patients remained hospitalized for a further 24 h following catheter removal, and were then monitored weekly for 4 weeks with physical examination, toxicity notation, and blood studies monitoring CBC, liver function, and renal function. Response to therapy was measured by computed tomography of the liver (4 patients) or liver-spleen scan (1 patient). Toxicity was quantified utilizing Southwest Oncology Group criteria, and response to therapy was judged by accepted standard definitions.

HPLC analysis for bisantrene. Plasma specimens were analyzed for bisantrene using a modification of the HPLC technique developed by Wu and Nicolau [16]. The analog of bisantrene, CL 238,985 (American Cyanamid, Pearl River, NY) served as an internal standard for each specimen. One milliliter plasma was extracted with 2 ml chloroform/methanol (83 : 16) containing 1% 6 *N* HCl. The mixture was agitated, and the phases were separated by centrifugation at 2000 *g* for 5 min. The aqueous phase was extracted again with 3 ml chloroform and 100 µl 28% ammonium hydroxide. The mixture was agitated and separated under centrifugation. Then, 2.5 ml of the organic layer was evaporated to dryness under nitrogen and over a 37 °C water bath. The doubly extracted residue was reconstituted with 200 µl of the chromatographic mobile phase. The solution was agitated, and 50–100 µl of the solution was injected into a Tracor model 995 HPLC instrument using a Waters µ Bondapak C_{18} reverse-phase column (3.9 mm i.d. × 30 cm). Elution was accomplished with a 30 : 60 : 5 solution of acetonitrile : water : ammonium formate (V/V/V) delivered at a flow rate of 2 ml/min. Bisantrene was detected by UV absorbance at 260 nm wavelength. Retention times for bisantrene and CL 238,985 were 2.5 min and 4 min, respectively. The detection limit for this assay is 20 ng/ml with a coefficient of variation of 10% or less over a concentration range of 20 ng/ml to 2 µg/ml.

Results

Patient characteristics

Five patients were treated with sequential hepatic arterial and peripheral vein infusions of bisantrene. Patient characteristics are summarized in Table 1. Four patients received only one course of treatment; a fifth patient received two. Four patients had normal arterial anatomy, a fifth patient had the gastroduodenal artery branching off the right hepatic artery distal to the separation of right and left hepatic arteries. Of the patients with normal anatomy, the common hepatic artery was cannulated in two, and the right hepatic artery was cannulated in two. In addition, only the right hepatic artery was cannulated in the patient with the distal branch of the gastroduodenal artery. Pretreatment liver functions and an estimate of malignant replacement of the liver from scan data are indicated for each patient in an attempt to correlate with the observed pharmacokinetic parameters.

Pharmacokinetic analysis

Blood sampling for bisantrene pharmacokinetic analysis was completed during the first course of treatment for all five patients. The data for patient 4 are the results of a single HPLC determination and vary substantially from the data of the other patients. Consequently, the pharmacokinetic data for this patient are excluded from further analysis. The results for the remaining four patients are considered.

Table 1. Patient characteristics

Patient	Age	Diagnosis	Pretreatment SGOT[a]	Alkaline phosphatase[a]	%Replacement of Liver by malignant disease	Arterial cannulation	Prior therapy
1	71	Hepatocellular carcinoma	187	261	>50%	Common hepatic a.	None
2	60	Adenocarcinoma, unknown primary	86	216	<50%	Right hepatic a.	None
3	63	Colorectal carcinoma	35	180	<50%	Right hepatic a.	5-FU, semustine vincristine
4	59	Colorectal carcinoma	52	145	<50%	Right hepatic a.; Left hepatic a. ligated	5FU,High-dose melphalan
5	65	Adenocarcinoma, unknown primary	48	440	>50%	Common hepatic a.	None

[a] Normal values: SGOT ≤ 8–40 IU/l; Alkaline phosphatase ≤ 30–115 IU/l

Table 2. Hepatic extraction of bisantrene during peripheral vein infusion

Patient	Total Dose (130 mg/m^2 × body surface area)	Duration of Infusion	EHPF (l/min)[a]	AUC[b] HA	AUC[b] HV	Hepatic extraction ratio[c]	Hepatic plasma clearance[d]
				(μg-h/l)			
1	231 mg	4 h	0.54	3666	3035	0.17	0.054 l/min/m^2
2	221 mg	4 h	0.82	2413	1629	0.33	0.159 l/min/m^2
3	268 mg	4 h	1.49	3860	1982	0.49	0.353 l/min/m^2
5	215 mg	2 h	0.31	1640	1388	0.15	0.029 l/min/m^2

[a] Estimated hepatic plasma flow calculated by indocyanine green infusion (see *Materials and methods)*
[b] Area under concentration-time curve; HA hepatic arterial; HV, hepatic venous
[c] Hepatic extraction ratio (HER) = $(AUC_{HA} - AUC_{HV}/AUC_{HA})$
[d] Hepatic plasma clearance = EHPF · HER

Table 3. Peripheral vein plasma bisantrene concentrations during hepatic arterial or peripheral vein infusion

Patient	Total dose by each infusion route	Duration of infusion	Hepatic arterial infusion		Peripheral vein infusion	
			Mean PV[a] plasma concentration (μg/l ± SD[b])	PV AUC[c] (μg · h/l)	Mean PV plasma concentration (μg/l ± SD)	PV AUC (μg · h/l)
1	231 mg	4 h	401 ± 62	1535	936 ± 72	3537
2	221 mg	4 h	334 ± 79	1292	729 ± 30	2733
3	268 mg	4 h	305 ± 40	1151	875 ± 315	4000
5	215 mg	2 h	765 ± 59	1338	1522 ± 323	2586

[a] PV – peripheral vein
[b] Standard deviation from the mean
[c] Area under the curve

HERs and hepatic plasma clearances were calculated from plasma concentrations generated during peripheral vein infusion of bisantrene. The following assumptions were made during these calculations: (1) hepatic arterial and portal venous concentrations are the same during infusion; (2) cannulation of either right or left hepatic vein provides a satisfactory representation of hepatic venous bisantrene concentrations during peripheral vein drug infusion. Bisantrene plasma concentrations, peak concentrations and areas under the concentration-time curve (AUCs) at all blood sampling sites were calculated for hepatic arterial and peripheral vein drug infusion. The hepatic extraction data are summarized in Table 2. It is notable that the HER is low for this anticancer agent, ranging from 0.17 to 0.49 (hepatic plasma clearance from 0.029 l/min/m^2 to 0.353 l/min/m^2). When reviewed with respect to pretreatment parameters of hepatic function or degree of metastatic involvement of the liver, these data suggest a rough correlation between higher hepatic extraction and less metastatic involvement or lower SGOT/alkaline phosphatase values.

Table 3 can compares the mean peak peripheral vein plasma bisantrene concentration and the peripheral vein plasma concentration-time AUCs for peripheral vein infusion and for hepatic artery infusion of the drug. Plasma bisantrene concentrations had returned to 60 μg/l or less (mean 10.6 μg/l) 24 h after the beginning of each drug infusion. Hepatic arterial infusion of bisantrene produces peripheral vein concentrations ranging from 35% to 50% of peripheral vein concentrations during peripheral vein bisantrene infusion. Similarly peripheral vein bisantrene concentration-time AUCs during hepatic arterial infusion

are 29%–52% of the AUCs during peripheral vein infusion. These data suggest a reduced yet substantial systemic exposure to bisantrene during regional infusion.

Toxicity and response

Four patients completed only one 28-day course of treatment. Patient no. 4 received two courses of treatment. None of the patients experienced a response to treatment in measurable liver metastases.

Toxicity of the therapy was limited to transient fever and myelosuppression. All patients developed leukopenia (median WBC nadir 2400/mm^3; range 1400–2700/mm^3) within 6–12 days of initiation of treatment. Thrombocytopenia and anemia were not observed. Patients 1 and 3 experienced fever of 14 days' and 2 days' duration, respectively. Patient 1 required parenteral antibiotics when leukopenia was superimposed upon fever. Infection was not detected in either case. No patient developed hypotension with any course of treatment, and no additional toxicities were noted. No clinical or biochemical evidence of drug-induced toxicity was noted in any patient. Recannulation of the hepatic artery was attempted in patient 2. Although catheterization was technically unsuccessful, hepatic arteriography revealed patency of the vessel without evidence of any detrimental effect of the prior bisantrene infusion.

Discussion

Based on phase II and phase III studies, bisantrene appears to have potential utility in the treatment of several malignancies, most notably breast cancer [13]. The administration of the agent is somewhat complicated by its poor solubility in human plasma and its risk of producing troublesome hypotensive events and venous phlebitis. A large apparent volume of distribution and high RBC concentrations of the drug suggested in animal studies indicate substantial tissue sequestration of bisantrene [16]. The prolonged terminal half-life of bisantrene in plasma (mean $t_{1/2}\gamma$ = 26.13 h) also supports very slow elimination of the drug from the body.

Identification of drugs suitable for hepatic arterial infusion requires recognition of hepatic extraction and hepatic clearances favoring a therapeutic advantage either for tumor exposure to the agent or for systemic exposure commensurate with reduced toxicity. Chen and Gross characterized the quantitative pharmacokinetic features of intraarterial therapy and provided derivations which describe the advantage of increased local delivery and decreased systemic delivery of antineoplastic agents [4]. With the pharmacokinetic data derived from this study, the relative advantage (R_t) to administration of bisantrene to the tumor-bearing site is:

$$R_t = 1 + \frac{(apparent\ plasma\ clearance)}{(tumor\ plasma\ flow)}$$

$$R_t = 1.09\text{–}1.24,$$

whereas the relative advantage (R_s) to the systemic delivery of drug following intraarterial infusion (and consequently the risk of systemic toxicity which may be anticipated) is:

$$R_s = \frac{1}{(1\text{-}HER)}$$

$$R_s = 1.18\text{–}1.96.$$

Both derived values are low and suggest little systemic advantage to intraarterial infusion of bisantrene versus peripheral vein infusion and only a modest advantage to the tumor-bearing organ with intraarterial administration. Although the observed toxicity in this study was moderate, the technical difficulties which may be anticipated with long-term intraarterial bisantrene infusion (risk of extravasation, hypotension, phlebitis, etc.) are worrisome. Regional infusion of bisantrene may be reserved for use in the treatment of malignancies against which phase II studies suggest activity (e.g., breast cancer). We intend to use this method of pharmacokinetic analysis to identify other new agents which may be appropriate for hepatic arterial infusion.

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