Cancer Chemother Pharmacol (1982) 9: 169–172

Cancer Chemotherapy and Pharmacology

Clinical Pharmacology of Bruceantin by Radioimmunoassay

K. L. L. Fong[1], D. H. W. Ho[1], R. S. Benjamin[1], N. S. Brown[1], A. Bedikian[1], B. S. Yap[1], C. L. Wiseman[1], W. Kramer[2], and G. P. Bodey[1]

[1] Department of Developmental Therapeutics, The University of Texas M.D Anderson Hospital and Tumor Institute at Houston, Houston, TX 77030

[2] Department of Pharmaceutics, College of Pharmacy, University of Houston, Houston, TX 77030, USA

Summary. *During the phase I clinical trial of a new antitumor agent, bruceantin, the pharmacology was studied in 18 cancer patients. The drug was infused intravenously (IV) for 3 h at doses ranging from 1 to 3.6 mg/m² per day for 5 days. The plasma drug disappearance curves were biphasic, with a fast initial half-life of less than 15 min. The second half-life ($t_{1/2\beta}$) varied from 0.7 to 38 h among different patients and was not dose-related. The difference between the $t_{1/2\beta}$ on day 1 and that on day 5 was not significant. In patients with normal liver function, the mean plasma concentration at the end of infusion was 22 ng/ml, and the value of the area under the concentration × time curve (AUC) was 111 (ng/ml)h. In contrast, in patients with abnormal liver function the corresponding values were 115 ng/ml and 830 (ng/ml)h, respectively. In addition, these patients had a slower elimination half-life of 10.9 h and a decreased total clearance of 157 ml/min/m², as compared with 2.6 h and 671 ml/min/m², respectively, for the normal group. All these differences were statistically significant.*

Patients with abnormal liver function developed more severe toxicity, including fever, severe nausea, vomiting, and hypotension. Two patients with severe hepatic dysfunction received a reduced dose and developed no toxicity. These results demonstrated the importance of the effects of liver dysfunction on drug disposition and showed that the dosage should be reduced in patients with hepatic dysfunction.

Introduction

Bruceantin (Fig. 1) is a plant product isolated from stem bark of *Brucea antidysenterica* [8, 9], which was used in Ethiopia for the treatment of cancer [7]. It irreversibly inhibits the initiation of protein synthesis in HeLa cells and rabbit reticulocytes, but has little effect on RNA synthesis [10]. Recent studies carried out in both HeLa cells and yeast by Fresno et al. have shown that bruceantin is a specific inhibitor of peptide bond formation [5]. Dissociation of polyribosomes to monoribosomes after bruceantin exposure (0.1 m*M*) has been reported [10]. However, a 10-fold higher concentration of bruceantin stabilized polyribosomes [10]. In preclinical toxicology studies

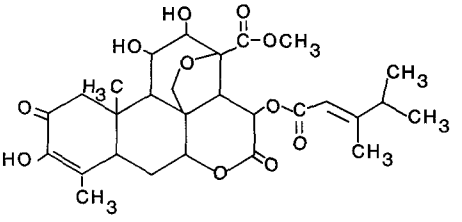

Fig. 1. Structure of bruceantin

in mice, dogs, and monkeys, bruceantin caused acute and chronic gastrointestinal and hepatic toxicity [2]. A specific microbiology assay was developed, and tissue disposition and metabolism of bruceantin were studied in both L1210 tumor-bearing and normal mice [15]. Bruceantin has shown significant antitumor activity against Walker 256 carcinosarcoma, Lewis lung carcinoma, B16 melanoma, P388 lymphocytic leukemia, and L1210 lymphoid leukemia [3, 8, 9]. Because of its broad spectrum of antitumor activity in experimental systems, clinical trials were initiated in patients with advanced neoplastic disease [1, 6]. After a radioimmunoassay had been developed [4], clinical pharmacology of bruceantin was studied in patients undergoing therapy with this drug at The University of Texas MD Anderson Hospital and Tumor Institute at Houston.

Materials and Methods

Bruceantin was supplied by the Developmental Therapeutics Program Chemotherapy, National Cancer Institute, Bethesda, MD, USA. The purity of the compound was checked by thin-layer chromatography, and only a single migrating spot was found [4]. Tritiated acetic anhydride (sp. act. 3.1 Ci/mmol) and [1,4-^{14}C]succinic anhydride (sp. act. 105 mCi/mmol) were obtained from Amersham/Searle, Arlington Heights, IL, USA. Dextran T-70 was purchased from Pharmacia, Uppsala, Sweden. Norit A charcoal was obtained from Fisher Scientific, Fair Lawn, NJ, USA. Other chemicals were commercially available.

Pharmacological studies were performed in 18 patients with metastatic cancer who were treated with bruceantin. Blood samples were collected on day 1, and whenever possible on day 5 for a total of 27 studies. The patients' characteristics and drug toxicities are shown in Table 1. Patients were divided into two groups. Those showing normal to mildly abnormal liver functions were grouped together because pharmacological parameters were not statistically different, and will be

Reprint requests should be addressed to D. H. W. Ho at the above address

Present address: K. L. L. Fong, Smith, Kline and French Research Laboratory, 1500 Spring Garden Street, Philadelphia, PA 19101, USA

Present address: C. L. Wiseman, Wilshire Oncology Medical Group, Inc., Los Angeles, CA 90057, USA

0344-5704/82/0009/0169/$ 01.00

Table 1. Dose, liver function, pharmacokinetics, and toxicity in individual patients

Pt no.	Dose mg/m^2 [e]	Rx day[a]	Liver Dysfunction	AP[b]	LDH[b]	SGOT[b]	BILI[b]	Liver scan[c]	C_p^0 ng/ml	$t_{1/2\beta}$ h	AUC (ng/ml)h	CL_T (ml/min/m^2)	$V_{d\beta}$ (l/m^2)	Toxicity, remarks[d]
1	3.6	C_1D_1	None	N	N	N	N	−	14	1.2	79	755	77	0, N, mild headache
		C_1D_5							18	2.7	63	957	225	
2	2.5	C_1D_1	None	N	N	N	N	−	14	1.2	48	871	89	0
		C_1D_5							32	1.2	319	131	14	
3	2.5	C_1D_1	None	N	N	N	N	−	16	1.1	48	879	83	0
4	3.3	C_1D_1	None	N	N	N	N	−	12	2.4	55	1014	212	0
5	3.5	C_1D_1	None	N	N	N	N	−	14	0.8	42	1384	90	0
		C_1D_5							23	2.2	87	659	124	
6	3.1	C_1D_1	None	N	N	N	N	−	14	2.4	66	888	185	0, Dying, expired next day
7	3.5	C_1D_1	Mild	165	N	N	N	−	14	6.6	145	357	202	0
8	3.5	C_1D_1	Mild	260	N	N	N	−	20	3.3	130	453	127	0
		C_1D_5							25	3.3	115	510	144	
9	2.1	C_1D_1	Mild	240	>600	N	N	−	67	1.6	224	178	24	0
10	2.5	C_1D_1	Mild	>350	N	N	N	−	31	4.8	216	308	129	BP↓
		C_1D_5							17	6.0	113	369	191	
	3.5	C_2D_1		>350	N	N	N	−	16	4.0	86	680	233	Tolerated well
11	3.6	C_1D_1	Mild	295	>600	N	N	−	35	3.9	135	442	148	0, N/V
12	3.5	C_1D_1	Mild	153	>600	71	N	−	8	2.1	42	1396	249	0
		C_1D_5							19	0.7	85	688	40	
		C_2D_1							36	1.4	118	510	60	
13	3.5	C_1D_3	Moderate	323	N	N	N	+	34	13.8	424	158	164	0 (Methadone)
		C_1D_5							23	5.9	202	303	156	
14	2.9	C_1D_1	Moderate	194	267	N	N	+	23	5.4	130	366	172	Severe fever & N/V hypotension, BP↓
15	3.5	C_1D_2	Moderate	244	379	N	N	+	82	2.1	375	156	28	N/V, BP↓
16	2.1	C_1D_1	Moderate	N	262	N	N	+	60	1.6	3838	15	2	Phlebitis, flushing, BP↓
17	1.0	C_1D_1	Severe	>350	>600	>300	>10	+	29	38.4	613	27	90	0, Dose reduced, expired 3 days p̄ BN
18	1.0	C_1D_5	Severe	>350	>600	>300	6	+	16	8.9	227	74	57	0, Dose reduced, expired 2 days p̄ BN

[a] C_1D_1, day 1 of 1st course of therapy, etc.
[b] N represents values within the normal ranges, which are listed in parentheses: AP, alkaline phosphatase (34–85 IU); LDH, lactate dehydrogenase (100–225 IU); SGOT, serum glutamic oxaloacetic transminase (7–40 IU); BILI, bilirubin (0–1 mg/dl)
[c] +, abnormal liver scan indicating liver metastases; −, negative (normal) liver scan
[d] N/V, nausea and vomiting; BP↓, drop in blood pressure; and p̄ BN, post bruceantin treatment
[e] Patients 1–12 are designated the normal group while patients 13–18 are designated the abnormal group

referred to as 'normal'. For the same reason, the second group of patients, which will be referred to as the 'abnormal' group, included those whose tests indicated moderate to severe hepatic dysfunction. Written informed consent was obtained from patients according to institutional policy.

Bruceantin, 1–3.6 mg/m^2/day for 5 days, was infused IV over 3 h. The drug was dissolved in 1.5 ml absolute ethanol and diluted with 500 ml 5% dextrose in 0.9% NaCl. Initially, heparinized blood samples were drawn before and after infusion at predetermined time intervals for 24 h. Because of the small therapeutic dose, the level frequently fell below the sensitivity (1 ng/ml) [4] of the assay after 7 h; therefore, the blood sampling was limited to 7 h and any results noted after that time were not included in the plots. Plasma was obtained after centrifugation and immediately frozen until assayed.

The radioimmunoassay for bruceantin used [^{3}H]acetyl-bruceantin and antibody induced by immunizing rabbits with succinylbruceantin-bovine serum albumin conjugate as described previously [4]. The assay was performed in 4-ml glass tubes containing 0.1 ml 0.01 *M* phosphate-buffered saline (PBS, 0.01 *M* NaH_2PO_4 and Na_2HPO_4 in 0.15 *M* NaCl with 0.1% NaN_3, pH 7.5), 0.1 ml antiserum diluted 1 : 10 with 0.1% gelatin in 0.01 *M* PBS to obtain a final dilution of 1 : 50 in the assay, [^{3}H]acetyl-bruceantin with approximately 2,500 cpm, and appropriate amounts of bruceantin standard (ranging from 0.5 to 50 ng) or unknown plasma in a total volume of 0.5 ml. The binding reaction was initiated by incubating at room temperature for 1 h after adding antiserum. The tubes were then placed in an ice bath and 0.5 ml of an ice-cold solution of 4% charcoal and 0.6% dextran in 0.01 *M* PBS was added. The assay was left standing in the ice bath for 30 min, with mixing at 10-min intervals. After centrifugation, the radioactivity in 0.5 ml of the supernatant was measured by adding 10 ml 66% Phase Combining System (Amersham/Searle Corp., Arlington Heights, IL) counting solution in xylene and counting in a Packard Tricarb Scintillation Spectrometer Model 3375. Any quenching was corrected by the channel ratios with an automatic external standard, and the counting efficiency for ^{3}H was 35%. Data points were determined for the standard curve in duplicate and for unknown samples in triplicate.

The data was analyzed with a Hewlett-Packard HP-65 programmable calculator using the exponential curve fit program. After the slopes of the curves were determined, the half-lives of the distribution phase ($t_{1/2\alpha}$) and elimination phase ($t_{1/2\beta}$) were calculated. Using the method of Loo and Riegelman [12], corrections for infusion times were made and

the total clearance (Cl_T), volume of distribution by the β method ($V_{d\beta}$) and area under the concentration versus time curve (AUC) were calculated [13]. The general blood level equation method was used for calculating AUC [13]. The Mann-Whitney test was used for statistical analyses between the normal and abnormal groups [14].

Results

The plasma drug disappearance curves following the first dose of bruceantin were biphasic. A representative graph from the group of patients with normal liver function is shown in Fig. 2, left panel. The $t_{1/2\alpha}$ was approximately 15 min and $t_{1/2\beta}$ was 70 min. The $t_{1/2\beta}$ result obtained on day 5 for one patient resembled that recorded on day 1 for the same patient (Fig. 2, right panel). Using the paired t-test [14], statistical analysis of the $t_{1/2\beta}$ of the six patients in the normal group who were studied on days 1 and 5 showed no significant difference ($P > 0.2$) between the two days. As shown in Table 1, which listed the pharmacokinetic parameters of the individual patients, the $t_{1/2\beta}$ was not affected by the various amounts of drug administered. For example, in the 12 studies with 3.5 mg/m^2 doses, the $t_{1/2\beta}$ ranged from 0.7 to 13.8 h and overlapped with those of other doses. The plasma disappearance curve (Fig. 3, right panel) of a patient in the group with abnormal liver function is compared with that of a patient who had normal liver function (Fig. 3, left panel). The plasma concentration at the end of infusion (C_p^0), even with a reduced dose, was higher and the plasma $t_{1/2\beta}$ was longer in the abnormal patient. Table 2 summarizes the individual data and statistically compares the normal and abnormal groups. The Cl_T in the patients with impaired liver function was smaller, resulting in a significantly higher value of AUC. Even when the AUC is normalized to a standard dose of 1 mg/m^2, there is a significant difference ($P < 0.002$) between the groups.

In seven patients, urine samples were collected for 22 h after the first bruceantin dose only. The percentage excreted was 10.4% ± 4.5% (mean ± SD) of the given dose. A representative graph of the cumulative urinary excretion is shown in Fig. 4.

A cerebrospinal fluid (CSF) sample was obtained from one patient 6 h after a bruceantin dose of 3.6 mg/m^2. No drug could be detected in this CSF sample, although the plasma collected at the same time contained 6.1 ng/ml.

Discussion

Phase I clinical trials of bruceantin have revealed that the dose-limiting toxicity is hypotension, which occurred more often in patients with abnormal liver function [1, 6, 11]. Our pharmacological studies of bruceantin indicate that patients in the abnormal group had an altered drug disposition which resulted in significant changes of C_p^0, $t_{1/2\beta}$, AUC, and Cl_T. The difference between the AUC of the normal and abnormal groups is particularly notable. Although the mean dose given to the abnormal group was smaller than that received by the normal group, the AUC was significantly larger in the abnormal group. Even with doses normalized, this relationship remained significant and emphasized the importance of hepatic function for bruceantin pharmacokinetics. Preliminary pharmacology performed in dogs demonstrated that biliary excretion was involved in bruceantin disposition [4]. If biliary excretion also occurred in man, alteration of pharmacokinetic parameters would be expected in patients with abnormal liver

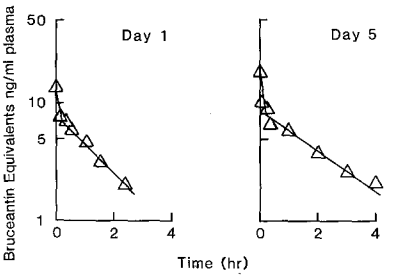

Fig. 2. Plasma drug disappearance curves of a patient after receiving a 3-h IV infusion of bruceantin (3.6 mg/m^2). *Left panel*, day 1; *right panel*, day 5

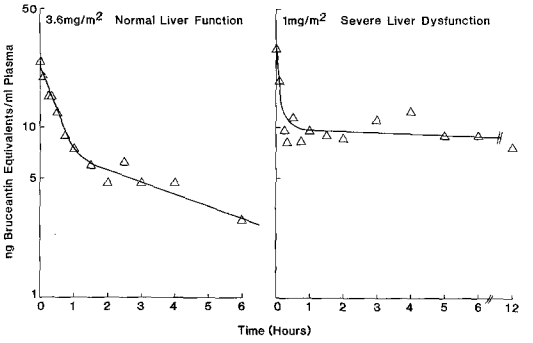

Fig. 3. Comparison of plasma drug disappearance curves of patients with normal (*left panel*) and abnormal (*right panel*) liver function

Table 2. Summary of pharmacokinetics of patients with normal and abnormal liver function

Liver Function	C_p^0 ng/ml	$t_{1/2\beta}$ h	AUC (ng/ml) h	Cl_T ml/min/m^2	$V_{d\beta}$ l/m^2
Normal	22.2 ± 3.0[a]	2.6±0.4	111 ± 16	671 ± 79	182 ± 53
Abnormal	115.3 ± 81.2	10.9±4.8	830 ± 505	157 ± 51	96 ± 26
P value[b]	< 0.02	< 0.02	< 0.002	< 0.002	> 0.2

[a] Mean ± SE
[b] P value is derived from the Wilcoxon rank test [14]

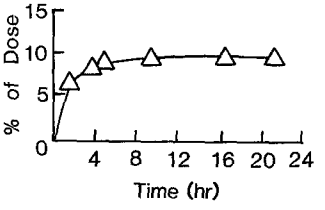

Fig. 4. Cumulative urinary excretion of a patient after receiving bruceantin (3.5 mg/m^2)

functions. Suling et al. [15] have shown that bruceantin is inactivated by an NADPH-dependent enzyme system present in mouse liver. This could imply that patients with liver dysfunction may metabolize bruceantin differently from those patients with normal liver function. However, because of the low bruceantin dose and the resulting low plasma concentrations, chromatographic identification of the parent compound or any metabolite was not possible.

Of the 12 patients in the normal group (6 with normal hepatic function and 6 with mild hepatic dysfunction), only one

had decreased blood pressure. He tolerated the second course well, even with an increase in dosage from 2.5 to 3.5 mg/m^2. Two other patients had slight nausea accompanied by vomiting and headaches. However, three of four patients with moderately abnormal liver function experienced hypotension, high fever, phlebitis, and nausea and vomiting. In contrast, two patients (# 17 and # 18) with severe hepatic dysfunction who received a reduced dose of bruceantin showed no toxicity. These results coupled with the pharmacokinetic findings suggest that bruceantin would be best given by individualized regimens. Unfortunately, the drug has yet to demonstrate any antitumor activity in phase II studies. These studies are now closed and further clinical studies with a better dose schedule are unlikely to be rewarding. Our study has demonstrated the importance of the effects of liver disease on drug disposition. Because of the wide variability, particularly in cancer patients, the design of the drug therapy should be individualized on the basis of pharmacokinetics [16].

Acknowledgements. The work reported in this paper was supported by grant CA 05831, and contract CM 87233, Division of Cancer Treatment, National Institutes of Health, Department of Health and Human Services.

We would like to thank Mrs. Sylvia Rose for her assistance in editing our manuscript.

References

1. Bedikian AY, Valdivieso M, Bodey GP, Freireich EJ (1979) Initial clinical studies with bruceantin. Cancer Treat Rep 63: 1843
2. Castles TR, Bhandari JC, Lee CC, et al. (1976) Preclinical toxicologic evaluation of bruceantin (NSC 165563) in mice, dogs, and monkeys. U.S. National Technical Information Service, Springfield (PB-257175), p 348
3. Douros J, Suffness M (1978) New natural products of interest under development at the National Cancer Institute. Cancer Chemother Pharmacol 1: 91
4. Fong KLL, Ho DHW, Carter CJK, Brown NS, Benjamin RS, Freireich EJ, Bodey GP Sr (1980) Radioimmunoassay for the detection and quantitation of bruceantin. Anal Biochem 105: 281
5. Fresno M, Gonzales A, Vazques D, Jimenez A (1978) Bruceantin, a novel inhibitor of peptide bond formation. Biochim Biophys Acta 518: 104
6. Garnick MB, Blum RH, Canellos GP, Mayer RJ, Parker L, Skarin AT, Li FP, Henderson C, Frei E III (1979) Phase I trial of bruceantin. Cancer Treat Rep 63: 1929
7. Hartwell JL (1976) Types of anticancer agents isolated from plants. Cancer Treat Rep 60: 1031
8. Kupchan SM, Britton RW, Ziegler MF, Sigel CW (1973) Bruceantin, a new potent antileukemic simaroubolide from *Brucea antidysenterica.* J Org Chem 38: 178
9. Kupchan SM, Britton RW, Lacadie JA, Ziegler MF, Sigel CW (1975) The isolation and structural elucidation of bruceantin and bruceantinol, new potent antileukemic quassinoids from *Brucea antidysenterica.* J Org Chem 46: 648
10. Liao LL, Kupchan SM, Horwitz SB (1976) Mode of action of the antitumor compound bruceantin, an inhibitor of protein synthesis. Mol Pharmacol 12: 167
11. Liesmann J, Belt RJ, Haas CD, Hoogstraten B (1981) Phase I study on bruceantin administered on a weekly schedule. Cancer Treat Rep 65: 883
12. Loo JCK, Riegelmann S (1970) Assessment of pharmacokinetic constants from post-infusion blood curves obtained after IV infusion. J Pharm Sci 59: 53
13. Ritschel WA (1976) Pharmacokinetics of single dose administration: In: Handbook of basic pharmacokinetics. Drug Intelligence, Hamilton, p 201
14. Sokal RR, Rohlf, FJ (1969) Assumption of analysis of variance: In: Biometry, the principles and practice of statistics in biological research. W. H. Freeman, San Francisco, p 391
15. Suling WJ, Woolley CW, Shannon WM (1979) Disposition and metabolism of bruceantin in the mouse. Cancer Chemother Pharmacol 3: 171
16. Wilkinson GR, Schenker S (1975) Drug disposition and liver disease. Drug Metab Rev 4: 139

Received March 12/Accepted June 1, 1982