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Protein binding of brequinar in the plasma of healthy donors and cancer patients and analysis of the relationship between protein binding and pharmacokinetics in cancer patients

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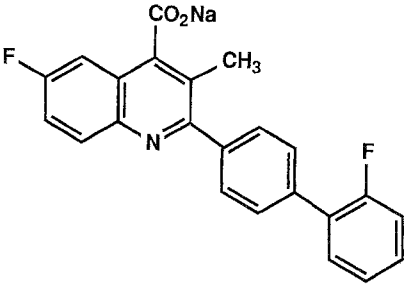
Abstract The protein binding of weakly acidic and basic drugs has been shown to be altered in cancer patients. Brequinar is a weakly acidic, low-clearance, and highly protein-bound (>98% bound) antitumor agent. The pharmacokinetic parameters of brequinar are subject to large interpatient variability. This large interpatient variability may be related to brequinar's plasma protein-binding capacity (assuming no change in the intrinsic clearance of the unbound drug). The objectives of this study, therefore, were (a) to characterize brequinar's protein binding in the plasma of healthy donors and cancer patients and (b) to examine the relationships between brequinar's plasma protein binding and its pharmacokinetics in patients. Brequinar protein binding was determined in human serum albumin (HSA) solution, drug-free donor plasma, and brequinar-free, predose plasma samples obtained from a phase I cancer trial. Pharmacokinetic results from this study were used to examine relationships between plasma protein binding and drug disposition. In HSA solution and healthy donor plasma, brequinar's protein binding as determined using spiked samples was concentration-dependent. The unbound brequinar fraction increased by a factor of 3 (from 0.3% to 0.9% free) in 4% HSA solution and by a factor of 4 (from 0.4% to 1.6% free) in donor plasma as the brequinar concentrations increased from 0.1 to 2.3 m*M* in the HSA solution and from 0.076 to 1.5 m*M* in the donor plasma. Analysis of brequinar binding characteristics using the binding ratio and Rosenthal binding plots showed that albumin was the primary protein for brequinar binding in human plasma. The addition of various concentrations of α_1-acid glycoprotein to 4% HSA solution did not affect the protein binding of brequinar to HSA. The protein binding determined in the plasma of cancer patients was not quantitatively different, except for variability, from that observed in the plasma of healthy donors. Examination of relationships between the unbound brequinar fraction and pharmacokinetics suggested that plasma protein binding was not a major determinant of brequinar disposition in cancer patients.

Key words Brequinar · Protein binding · Pharmacokinetics

Introduction

Brequinar sodium (DuP 785; Fig. 1) is a novel antitumor agent that has entered phase II clinical trials. Brequinar is an antimetabolite and exerts its antitumor activity by inhibiting dihydroorotate dehydrogenase, the fourth enzyme in the de novo pyrimidine biosynthetic pathway. This, in turn, depletes precursors for DNA and RNA synthesis [7]. The pharmacokinetics of brequinar after intravenous dosing have been examined in phase I trials and are characterized by a low total plasma clearance, a low renal clearance, a long terminal half-life, and a small

Fig. 1 Chemical structure of brequinar sodium (DuP 785)



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volume of distribution [3, 6, 15, 19]. All these pharmacokinetic parameters are subject to wide inter- and intrapatient variations. Brequinar is primarily cleared and excreted via the biliary/fecal route. Biotransformation studies conducted in the rat suggested that brequinar was primarily metabolized by the liver and eliminated in the bile (S. Diamond and D. Christ, unpublished data).

The influence of diseases on protein binding of drugs in either plasma or serum has been well documented [10, 14]. Altered protein binding characteristics in cancer patients have been reported for weakly acidic drugs, e.g., etoposide [20], apazone [21], and warfarin [21], and basic drugs, including lidocaine [12], propranolol [2], methadone [1], and imipramine [18]. It is therefore important to understand the protein-binding behavior of brequinar in cancer patients with normal hepatic and renal functions.

Brequinar's total plasma clearance (usually less than 50 ml/min) is much lower than the typical hepatic plasma flow (~800 ml/min for a 70-kg man [5]). Therefore, brequinar can be considered as a low-clearance drug and its elimination rate is not limited by liver blood flow [23]. Any alterations in brequinar's plasma protein binding, therefore, may cause changes in its total plasma clearance (assuming no significant change in the intrinsic clearance of the unbound drug). Correlations between the unbound fraction and pharmacokinetic parameters including total plasma clearance have been demonstrated for compounds such as warfarin [25, 27], bilirubin [17], dicumarol [28], phenytoin [11], tolbutamide [24], valproic acid [22], and sulfisoxazole [26]. Large interpatient variability in brequinar's pharmacokinetic parameters may be related to variability in the amount of plasma protein binding.

The objectives of this study, therefore, were (a) to characterize and compare brequinar's protein binding in human plasma collected from healthy donors and cancer patients and (b) to examine relationships between the unbound brequinar fraction and the drug's pharmacokinetics in cancer patients.

Materials and methods

Materials

Brequinar sodium was obtained from The DuPont Merck Pharmaceutical Company (Wilmington, Del.). The plasma from drug-free, healthy donors was purchased from Biological Specialty Co. (Lansdale, Pa.). The Centrifree micropartition system was purchased from Amicon Division, W.R. Grace & Co. (Danvers, Mass.). Human α_1-acid glycoprotein (α_1-AGP) and human serum albumin (HSA; Sigma, St. Louis, Mo.) were used as received.

Pharmacokinetics in cancer patients

Patients with solid tumors who had participated in a phase I trial were selected for this study [8]. This clinical trial was a nonrandomized, open label study. All patients had been off all previous chemotherapy or radiation therapy for at least 3 weeks. Each patient was scheduled to receive a single weekly intravenous dose of brequinar sodium for 4 consecutive weeks. Doses examined were 370, 490, 650, 860, 1140, and 1500 mg/m^2. After doses 1 and 4, blood samples for plasma (heparinized) preparation were obtained immediately before dosing, at the end of infusion, and at various times after the end of infusion. Urine and fecal samples were also collected after each dose.

Plasma, urine, and fecal samples were analyzed for intact brequinar using a high-performance liquid chromatography (HPLC) method with UV detection after an ion-pair liquid-liquid extraction procedure [3]. Unbound brequinar concentrations in ultrafiltrates of plasma samples obtained from two patients after administration of 1140-mg/m^2 doses were also determined using the HPLC method. The validated range of brequinar standards in biological matrices was 0.025–20 μg/ml. The plasma, urine, and fecal samples were diluted to assure that the concentrations were within the assay standard ranges. Pharmacokinetic parameters, including total plasma clearance (CL), renal clearance (CL_r), terminal half-life ($t_{1/2}$), mean residence time (MRT), and steady-state volume of distribution (V_{ss}), were calculated using model-independent methods [9]. The area under the plasma concentration versus time curve (AUC) was calculated using the linear trapezoidal rule.

Protein-binding determinations

The protein binding of brequinar in the plasma of healthy donors, in the plasma of cancer patients, and in solutions of α_1-AGP and HSA was determined by an ultrafiltration technique using the Centrifree micropartition system. The solutions of HSA and α_1-AGP were prepared in 0.07 *M* isotonic sodium phosphate buffer (pH 7.4). The patients' plasma samples used for protein-binding determination were brequinar-free, predose samples obtained from the clinical study.

In a test tube, brequinar sodium was mixed with 1.0 ml of plasma from healthy donors, 1.0 ml of patients' plasma, or 1.0 ml of solutions of HSA and α_1-AGP for a typical protein-binding determination. To examine concentration-dependent protein binding, brequinar concentration ranges of 0.076–1.5 m*M* in the plasma from healthy donors and of 0.1–2.3 m*M* in HSA were used. An aliquot (0.1 ml) of the plasma or protein sample was taken before centrifugation for determining brequinar total concentration. After incubation at 37° C for 20 min, the remaining 0.9 ml of the plasma or protein sample was transferred to the Centrifree micropartition system. The plasma sample was centrifuged at 3000 rpm (1000–2000 *g*) for 10 min with a fixed-angle rotor at 37° C to collect about 230 μl of the ultrafiltrate. Brequinar concentrations in plasma or protein solution and in ultrafiltrate were determined using an HPLC method [3]. The plasma or protein sample was diluted to ensure that the concentration of brequinar was within the assay standard range. The unbound brequinar fraction (f_p) was calculated by the ratio of the drug concentration in the filtrate to that in the plasma.

To examine whether brequinar would adsorb to the Centrifree device, brequinar (150 μg) was mixed with 1.0 ml of the ultrafiltrate before its placement into the ultrafiltration device. Brequinar's concentrations in the precentrifuged and postcentrifuged filtrates were similar, with the concentration ratio being 1.01 ± 0.03 (mean $\pm$SD, $n = 5$), suggesting that there was no nonspecific binding of brequinar to the Centrifree devices.

The binding characteristics were examined by the following methods. The relationship between the protein-binding ratio (bound versus free, B/F) and the albumin concentration was analyzed by the following equation:

$$\frac{B}{F} = nKP_T - K[B], \quad (1)$$

where n is the number of binding sites, K is the affinity constant, P_T is the protein concentration, and $[B]$ is the bound brequinar concentration. The binding parameters were also estimated using a model with two independent binding sites:

$$\frac{[B]}{[F]} = \frac{n_1P_1K_1}{1 + K_1[F]} + \frac{n_2P_2K_2}{1 + K_2[F]}, \quad (2)$$

where n_i is the number of ith-class binding sites per protein molecule, P_i is the protein concentration having the ith-class binding sites, K_i is the affinity constant(s) for the ith-class binding sites, and $[F]$ is the unbound or free brequinar concentration. Attempts made to fit the

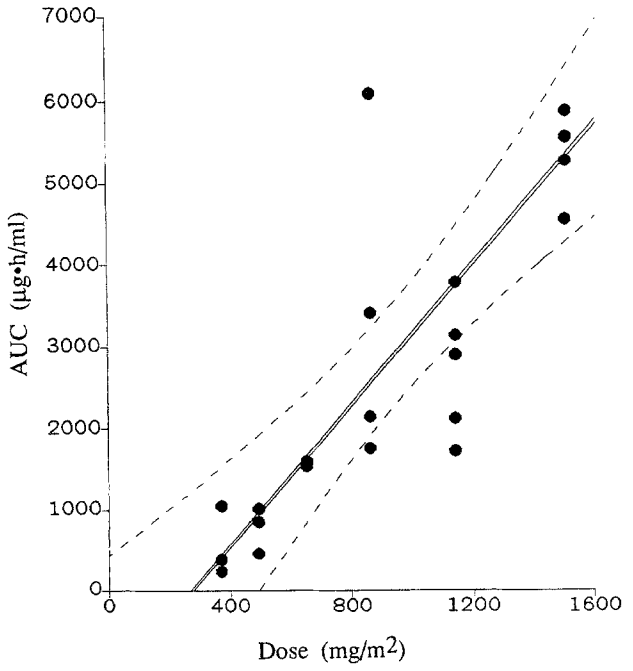


Fig. 2 Relationship of brequinar AUC versus dose after administration of the first weekly dose in cancer patients. The regression line and 95% confidence region are presented. The regression equation is AUC = 4.4 × (dose) − 1194, $r = 0.85$, $n = 23$ patients

binding data to the model equation (Eq. 2) using the nonlinear least-squares, Marquardt-Levenberg method failed. Therefore, the binding parameters (n_1P_1, K_1, n_2P_2, and K_2) were estimated by analyzing the data in the Rosenthal plots with the method of residuals.

Serum albumin and bilirubin

The serum albumin levels in samples from healthy donors were determined by the Biological Specialty Co. The serum albumin and bilirubin levels in samples from cancer patients were determined by the clinical study site (Memorial Sloan-Kettering Cancer Center, New York, N.Y.).

Table 1 Pharmacokinetic parameters[a] of brequinar after administration of doses 1 and 4 using a dose regimen of once-weekly intravenous doses for 4 weeks in cancer patients

Parameter	Dose 1 ($n = 23$)		Dose 4 ($n = 17$)[b]	
CL (ml/min)	14 ± 10	(71%)	14 ± 8	(57%)
CL_r (ml/min)	0.31 ± 0.19	(61%)	0.37 ± 0.27	(73%)
MRT (h)	17 ± 9	(53%)	16 ± 6	(38%)
$t_{1/2}$ (h)[c]	12 ± 5	(42%)	13 ± 4	(31%)
V_{ss} (l/kg)	0.16 ± 0.05	(31%)	0.17 ± 0.09	(53%)

[a] All data are expressed as mean values ± SD (coefficient of variation)
[b] The fourth weekly doses were not given to 6 patients
[c] Terminal half-life is expressed as the harmonic mean value ± pseudo-SD [13]

Statistical analyses

Statistical analyses were performed with the RS/1 statistical package (Release 3.0 Software, BBN Software Products Co., Cambridge, Mass.). A two-sample t-test was used to test for significant differences between two groups at a significance level of 5% ($P < 0.05$). Linear correlation analysis was used to test for statistically significant correlations between two parameters at the 5% level ($P < 0.05$).

Results

This paper reports only the pharmacokinetic results obtained from the phase I clinical study. Detailed safety results have been presented elsewhere [8]. The pharmacokinetics of brequinar were characterized after the administration of single weekly intravenous doses for 4 weeks in cancer patients. Brequinar disposition was linearly related to the dose over the examined dose range of 370–1500 mg/m^2 (Fig. 2). The AUC-versus-dose data were also tested using two second-degree polynomial functions as nonlinear functions. The intercept in one of the two polynomial functions was set to zero. Results from nonlinear regres-

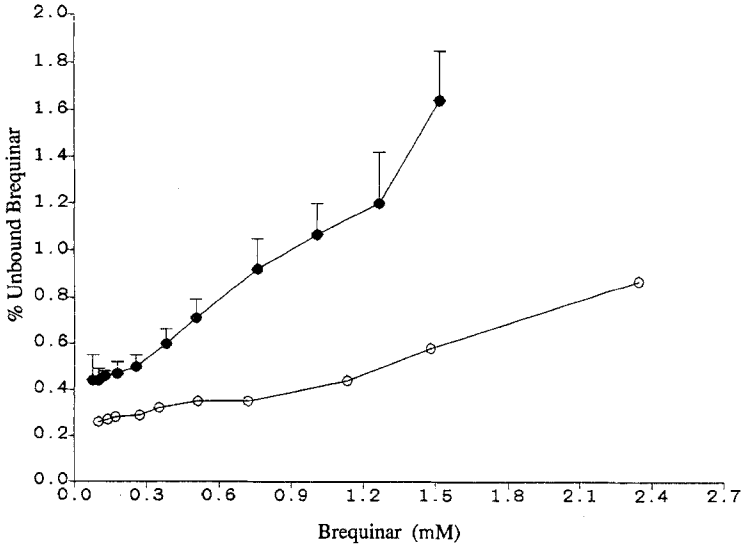


Fig. 3 Brequinar protein binding versus total brequinar concentration as determined in the plasma of healthy donors (●; mean values + SD, $n = 5$ donors) and 4% HSA (○; mean values for 4 determinations) at 37° C

Table 2 Albumin concentration and characteristics of brequinar protein binding as determined in HSA solution and in heparinized plasma of healthy donors and cancer patients[a] (*CV* Coefficient of variation, *NA* not available)

Variable	Healthy donors (n = 11)	Cancer patients		HSA
		Dose 1 (n = 23)	Dose 4 (n = 17)	
Albumin (g/dl)	4.2 ±0.3	4.0 ±0.4	4.0±0.4	4.0
% Unbound brequinar in plasma or HSA	0.65±0.18 (CV, 28%)	0.64±0.40 (CV, 63%)	0.60±0.25 (CV, 42%)	0.29±0.01[b]
nk (10^5 M^{-1})[c]	NA	7.9	7.2	5.5
n_1P_1 (mM)	0.47±0.16[d]	NA	NA	0.32
K_1 (10^5 M^{-1})	3.1 ±2.3[d]	NA	NA	3.5
n_2P_2 (mM)	2.5 ±0.3[d]	NA	NA	3.6
K_2 (10^4 M^{-1})	6.5 ±1.6[d]	NA	NA	8.9

[a] All data are expressed as mean values ±SD. The spiked brequinar concentration was 0.25 mM
[b] Average value for 4 determinations at spiked brequinar concentrations of 0.27 mM
[c] The product of the number of binding sites and the affinity constant
[d] Average values for 5 donors

sion analyses did not support the existence of a nonlinear relationship between AUC and dose.

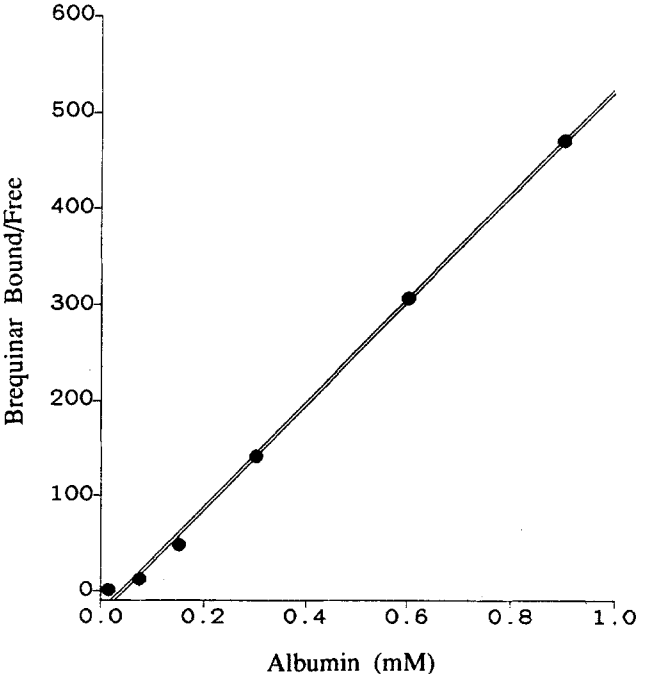
Mean values obtained for relevant pharmacokinetic parameters after doses 1 and 4 are shown in Table 1. All patients possessed normal hepatic (serum bilirubin, <1.5 mg/dl) and renal (serum creatinine, <2.0 mg/dl) functions. Brequinar's pharmacokinetic parameters in this patient population were subject to very wide interpatient variations that, expressed as the coefficients of variation of the mean values, ranged from 31% to 73% (Table 1). Consistent with the results reported in other phase I trials [3, 6, 15, 19], brequinar's pharmacokinetics were also characterized by a low CL, a low CL_r, a small V_{ss}, a long MRT, and a long $t_{1/2}$ in our patient population. The mean pharmacokinetic parameters obtained after dose 1 were similar to those obtained after dose 4. The CL_r accounted for less than 5% of the total plasma clearance. The total amounts of intact brequinar excreted in urine and feces from zero to 72 h were 2.7%±1.3% (mean ±SD, n = 22) and 15%±12% (n = 15) of the dose, respectively. This result suggests that the biliary/fecal route is a more important pathway than the renal route for brequinar elimination.

Brequinar was highly bound (>99%) to plasma proteins and to HSA (Table 2). The unbound brequinar fraction determined in the plasma of healthy donors was similar to that determined in patients' plasma (Table 2). However, the interindividual variability in the unbound brequinar fraction was 1.5–2 times greater in patients' plasma than in the plasma of healthy donors. The serum albumin levels also did not differ between patients and healthy donors (Table 2).

In spiked plasma samples from healthy donors and in HSA samples, brequinar protein binding at an albumin concentration of 0.6 mM was concentration-dependent over the examined concentration range of total brequinar (Fig. 3). At the equimolar concentration of brequinar and HSA, the unbound brequinar fraction was 0.35%. As the brequinar concentration increased from 0.1 to 2.3 mM in 4% HSA solution, there was a 3-fold increase from 0.3% to 0.9% in the unbound brequinar fraction. Brequinar protein binding in the plasma of healthy donors showed a similar trend in binding behavior. There was a 4-fold increase in the unbound brequinar fraction from 0.4% to 1.6% as the brequinar concentration increased from 0.076 to 1.5 mM.

The binding characteristics of brequinar in plasma and HSA solution were examined using the correlation plot of the protein-binding ratio (bound/free) versus albumin concentration and the Rosenthal binding plot. A strong linear dependence of brequinar binding to HSA was observed (Fig. 4). A significant linear correlation (P<0.05) between the brequinar binding ratio and the albumin concentration was also observed in the plasma of cancer patients after administration of doses 1 (data not shown) and 4 (Fig. 5).

Fig. 4 Correlation plot of the protein-binding ratio (*Bound/Free*) obtained for brequinar versus albumin in solutions of HSA. The regression equation is B/F = 545 × (albumin)−22, n = 6, r = 0.999. The spiked brequinar concentration was 0.27 mM



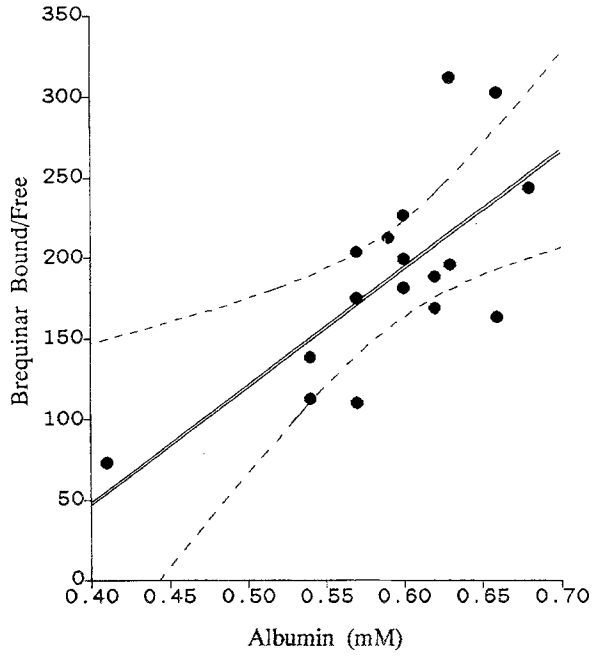


Fig. 5 Correlation plot of the plasma protein-binding ratio (*Bound/Free*) obtained for brequinar versus serum albumin after administration of the fourth weekly doses in cancer patients. The regression line and 95% confidence region are presented. The regression equation is B/F = 724 × (albumin)−242, n = 17 patients, r = 0.72, $P<0.05$. The spiked brequinar concentration was 0.25 m*M*

The slopes of these correlation plots (Figs. 4, 5) provided estimates of a binding parameter, nK, which is the product of the number of binding sites (n) and the affinity constant (K). The nK value obtained for brequinar in the HSA solution was similar to the values obtained in patients' plasma (Table 2).

The Rosenthal binding plots for brequinar as determined in a donor's plasma and in 4% HSA solution (Fig. 6) showed nonlinear binding relationships. The binding parameters of a model with two independent binding sites (n_1P_1, K_1, n_2P_2, K_2) are presented in Table 2. These binding parameters for brequinar binding to HSA were also similar to the binding parameters for brequinar binding to plasma proteins.

Table 3 Contribution of α_1-AGP to the overall brequinar protein binding observed in solutions of HSA and α_1-AGP

α_1-AGP[a, b]		% Unbound brequinar[c]
(mg/dl)	(μ*M*)	
0	0	0.87 ± 0.01
80	18	0.83 ± 0.01
150	34	0.85 ± 0.04
200	45	0.91 ± 0.15

[a] HSA concentration, 0.6 m*M*; brequinar concentration, 2.3 m*M*
[b] Brequinar binding to α_1-AGP was independent of the α_1-AGP concentration over a protein concentration range of 11–45 μ*M*. At a brequinar concentration of 0.024 m*M*, the proportion of brequinar that bound to α_1-AGP over this protein concentration range was 10% ± 6% (mean value ± SD, n = 15 determinations)
[c] All data are expressed as mean values ± SD for 4 determinations

The role of brequinar protein binding to α_1-AGP in a solution of α_1-AGP and HSA was assessed (Table 3). The binding of brequinar to α_1-AGP was not extensive (about 10% protein-bound) and was independent of the α_1-AGP concentration. At a brequinar concentration of 2.3 m*M*, α_1-AGP concentrations were varied from 0 to 45 μ*M* (0–200 mg/dl) for assessing the effect of α_1-AGP on brequinar binding in a 4% HSA solution. The average α_1-AGP levels measured in the plasma of healthy subjects and cancer patients are 80 and 140 mg/dl, respectively [2, 12]. If α_1-AGP makes a significant contribution to the overall brequinar binding in a mixed environment of HSA and α_1-AGP, an increase in the α_1-AGP concentration should decrease the unbound brequinar fraction. The results shown in Table 3 indicated that the increase in α_1-AGP

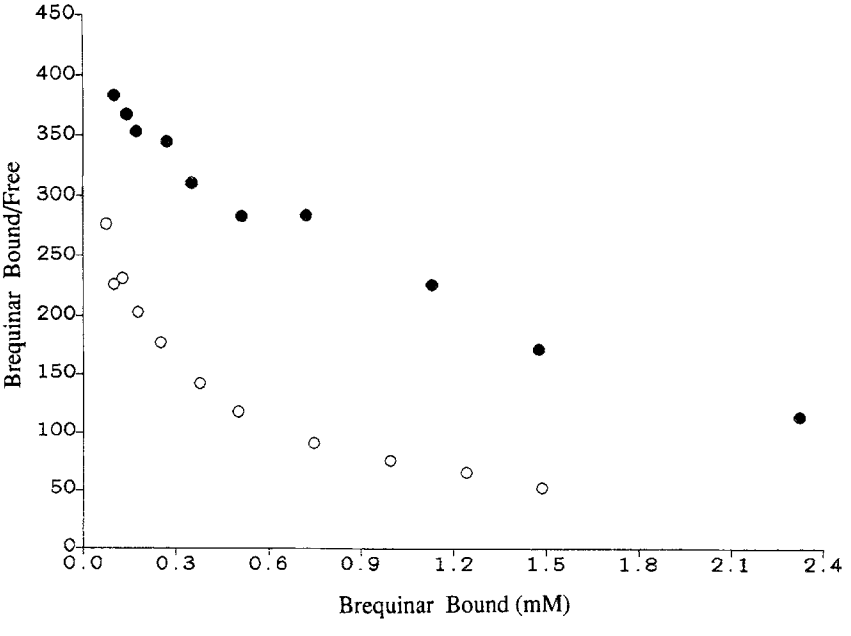


Fig. 6 Rosenthal binding plot for brequinar as determined in heparinized plasma of donor 1380 (○) and 4% HSA (●) at 37° C

Table 4 Summary of linear correlation analysis of the percentage of unbound brequinar or serum chemistry versus brequinar pharmacokinetic parameters in cancer patients (*NS* Not significant)

Parameter	Dose 1 (n = 23)	Dose 4 (n = 17)
f_p vs CL (ml/min)	NS	NS
f_p vs CL (ml min^{-1} kg^{-1})	NS	NS
f_p vs CL_r	NS	NS
f_p vs V_{ss}	NS	NS
f_p vs $t_{1/2}$	NS	NS
f_p vs MRT	NS	NS
CL (ml/min) vs bilirubin	NS	$r = 0.53$, $P < 0.05$
CL (ml min^{-1} kg^{-1}) vs bilirubin	NS	$r = 0.61$, $P < 0.05$
CL (ml/min) vs albumin	NS	NS
CL (ml min^{-1} Kg^{-1}) vs albumin	$r = 0.46$, $P < 0.05$	NS

concentration did not affect brequinar protein binding in a 4% HSA solution.

A linear correlation analysis was performed to examine relationships between the unbound brequinar fraction (f_p) versus CL, CL_r, $t_{1/2}$, MRT, and V_{ss} (Fig. 7, Table 4). The CL seemed to increase with the unbound drug fraction, but the correlation was not statistically significant ($P > 0.05$; Fig. 7). The unbound fraction was also not correlated with CL_r, MRT, $t_{1/2}$, or V_{ss}. Relationships of CL versus serum bilirubin and albumin levels were also examined to evaluate the utility of these two serum parameters as markers of the brequinar clearance rate in patients (Fig. 8, Table 3). A weak but linear ($P < 0.05$) correlation was found between CL and bilirubin level. Brequinar's CL, when normalized by the patients' body weights, was found to have a weak but linear ($P < 0.05$) correlation with serum albumin level.

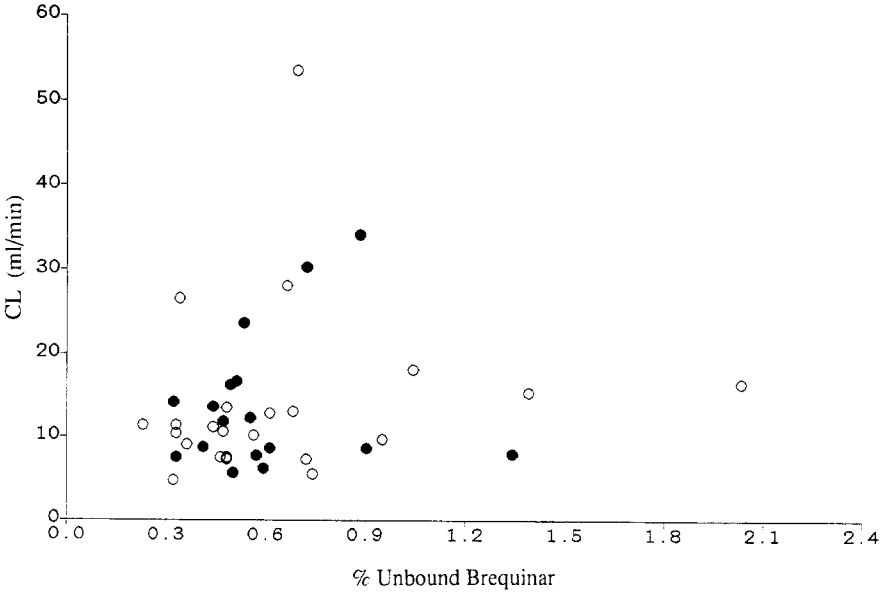


Fig. 7 Relationship between brequinar's clearance and the unbound brequinar fraction as determined in cancer patients following administration of the first (○) and fourth (●) weekly doses (each point represents one patient)

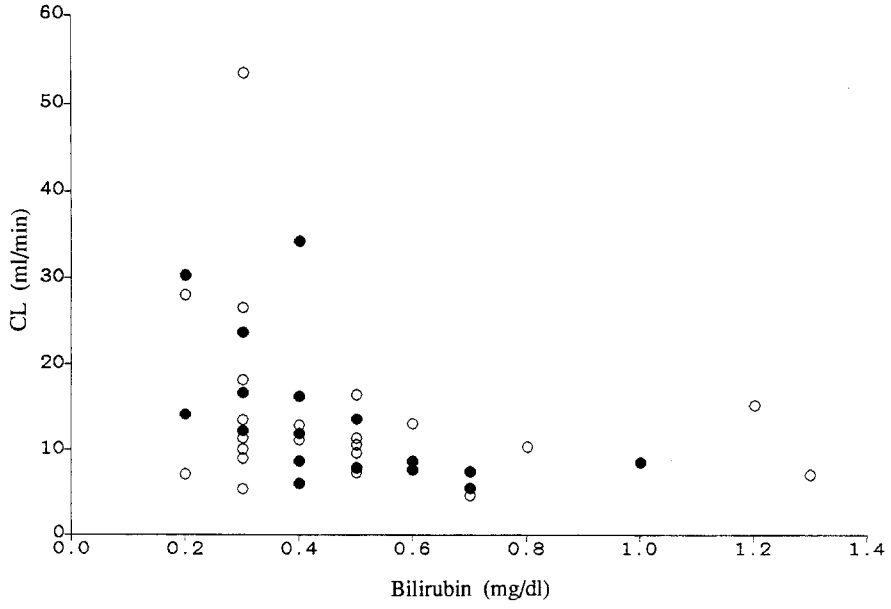


Fig. 8 Relationship between brequinar's clearance and the serum bilirubin concentration as determined in cancer patients following administration of the first (○) and fourth (●) weekly doses (each point represents one patient)

Discussion

In two earlier phase I trials [6, 19], a wider brequinar dose range of 36–2250 mg/m^2 was used to examine the pharmacokinetics. Because of the wider dose range, the AUC-versus-dose plot showed a nonlinear relationship between these two parameters, i.e., brequinar disposition was dose-dependent. After pooling of AUC-versus-dose data from all five phase I trials, the composite data indicated that brequinar's pharmacokinetics showed a dose-dependent threshold phenomenon at doses ranging from 36 to 2250 mg/m^2. The threshold dose appeared to be 200 mg/m^2. There were two linear segments in the AUC-versus-dose curve of brequinar: the first ranged from 36 to 200 mg/m^2 and the second, from 200 to 2250 mg/m^2.

The results of this study suggest that albumin is the primary protein for brequinar binding in human plasma. Albumin appears to possess two kinds of protein-binding sites for brequinar that include one high-affinity and low-capacity site and another site of lower affinity but very large binding capacity (probably four binding sites). Brequinar's binding to HSA was more extensive and less variable than its binding to plasma proteins. This discrepancy may be due to the presence of other endogenous and exogenous materials that may cause an increase in the unbound fraction of brequinar in human plasma.

The protein binding of some weakly acidic and basic drugs in patients' plasma has been shown to be affected by cancer [1, 2, 10, 12, 14, 18, 20, 21]. The primary changes due to the disease state are the increase in serum α_1-AGP concentration and the decrease in serum albumin concentration in patients with normal hepatic and renal function. The decrease in albumin concentration is due to increases in plasma volume and metabolic rate and to a decrease in the rate of protein synthesis [16]. Therefore, alterations in plasma protein binding of these drugs can be attributed to the disease-induced changes in concentrations of binding proteins. In our study population, the mean unbound brequinar fractions did not differ in the plasma of patients versus healthy donors because the serum albumin levels were similar. However, brequinar protein binding in patients' plasma was subject to larger interpatient variability. The brequinar binding was found to be linearly correlated ($P < 0.05$) with the serum albumin level in patients' plasma. This correlation indicates that the decrease in brequinar plasma protein binding is most likely due to the fewer total binding sites available in patients with hypoalbuminemia.

We showed that brequinar's protein binding as determined using spiked plasma samples from healthy donors was dependent on the drug concentration over a concentration range of 0.076–1.5 m*M*. However, the unbound brequinar fractions, determined in vivo in plasma samples obtained from two patients after intravenous administration of 1140-mg/m^2 doses, were not dependent on drug plasma levels. The unbound brequinar fraction determined in these patients' samples was 0.50% ± 0.13% (mean ± SD, $n = 36$; range, 0.3%–0.81%) over a brequinar concentration range of 0.043–1.3 m*M*. The reason for this difference between in vivo protein binding results obtained in cancer patients and the binding results obtained using spiked plasma samples from healthy donors is not known. One possible explanation is that the capacity of albumin-binding sites for brequinar may be altered in the plasma of cancer patients either because of the disease state or due to the presence of other concomitantly given drugs. These other drugs may bind to different sites in albumin and exert allosteric changes in the capacity of brequinar binding sites.

In a study by Aungst et al. [4], the effect of sodium salicylate (a known acidic drug that displaces the protein binding of other acidic drugs) administration on the intravenous pharmacokinetics of brequinar was examined in the rat. Brequinar protein binding in rat serum was decreased by about a factor of 2 in the presence of salicylate. After either oral or intravenous 10- and 50-mg/kg doses of salicylate, brequinar's CL and V_{ss} were significantly higher than those obtained for the control group, i.e., without salicylate treatment. These results indicate that at least in the rat, brequinar's clearance increases as the unbound drug fraction increases in rat plasma. In our cancer trial, the unbound brequinar fraction in patients' plasma was not a major determinant of brequinar disposition. This discrepancy in results obtained between rats and patients may be explained by the following two reasons. First, the lack of correlation between the unbound drug and CL and other pharmacokinetic parameters may be due to very large interpatient variations in both protein binding and pharmacokinetic parameters. Consequently, the sample size was not large enough to demonstrate protein binding as a determinant of brequinar disposition. Second, the intrinsic clearance of the unbound drug may be changed in cancer patients. If this were the case, brequinar metabolism could be a more important determinant of drug disposition. Brequinar's CL determined after the fourth brequinar dose was linearly correlated ($P < 0.05$) with serum bilirubin levels. These results seem to support the second possibility that metabolism or specifically intrinsic clearance may play a more important role in determining brequinar's pharmacokinetic properties.

In summary, the brequinar protein binding determined in our patient population was not different, except for variability, from that observed in the plasma of healthy donors. Brequinar protein binding was linearly related to serum albumin concentration in patients' plasma. Examination of relationships between the unbound drug fraction and the pharmacokinetic parameters suggested that plasma protein binding was not a major determinant of brequinar disposition in cancer patients.

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