Methadone plasma protein binding: Alterations in cancer and displacement from α_1-acid glycoprotein

Because of their elevated concentrations of plasma α_1-acid glycoprotein (AAG), cancer patients had a lower free fraction of methadone in plasma than did members of a control group. This difference was not great (−20%), but there was a fourfold variation in free fraction among a group of 13 patients (0.064 to 0.23). The bound/free methadone concentration ratio correlated linearly with plasma AAG. The binding of methadone to AAG was characterized by two classes of binding sites, the more avid having an association constant of $4 \times 10^5 M^{-1}$ and an N of 0.38. Methadone could be displaced from AAG binding sites by a number of drugs: propranolol, chlorpromazine, prochlorperazine, thioridazine, and imipramine. The concentrations required for significant displacement (27 μM), as well as the relatively low K_a for methadone, suggest that the free fraction of methadone will not be significantly affected by elevated methadone concentrations or through displacement by other drugs that also bind to AAG.

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It was reported by an attending physician that a cancer patient who had been receiving methadone over the long term for pain had experienced an unintentional narcotic-like overdose reaction without any change in methadone dose or schedule. Discounting any pharmacodynamic reason for this adverse reaction, the two aspects of drug disposition that might cause a drug to suddenly appear more potent than it had been would be inhibition of first-pass metabolism and decreased plasma protein binding. The first possibility seems unlikely since the bioavailability of oral methadone is over 90%.[10] On the other hand, the protein binding of methadone is known to be high, about 90%, and α_1-acid glycoprotein (AAG) is the main determinant of the free fraction in plasma.[16] Perhaps altered binding to AAG was in some way responsible.

We have reported that the elevations of AAG in cancer patients increase plasma binding as assessed by the test drug propranolol.[1] Although samples from the patient in question could not be obtained, we report here a second study in which methadone rather than propranolol was used. To further evaluate possible sources of altered methadone binding, we performed studies of the displacement of methadone from AAG and plasma for a variety of drugs known to bind to AAG.

Materials and methods

Our subjects were 13 patients (eight men; five women; 18 to 80 yr old) with advanced cancer who were entering a variety of clinical protocols of the National Cancer Institute. Five patients had sarcomas, four had Hodgkin's disease,

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Table I. *Plasma protein concentrations and methadone binding in cancer patients and control subjects*

	Cancer patients (n = 13)	*Control subjects (n = 11)*
Plasma AAG (mg/dl)		
Mean	151*	78
SD	53	21
Plasma albumin (gm/dl)		
Mean	3.08*	4.31
SD	0.51	0.42
Methadone free fraction		
Mean	0.194†	0.156
SD	0.044	0.040

*$P < 0.001$ compared to control; †$p < 0.05$ compared to control.

three had ovarian cancer, and one had a glioblastoma. A group of healthy subjects (seven men; four women, 28 to 55 yr old) served as controls. These populations are essentially the same as those previously studied,[1] although different samples from some individuals were required for the present study.

The techniques for sample acquisition and processing and the radial immunoassays for AAG and albumin have already been described.[1] The plasma protein binding method was also similar, except that plasma samples were spiked with tritiated methadone that was purified as follows. One hundred microcuries *l*-methadone hydrobromide (New England Nuclear, 161 mCi/mmol) containing tritium in the 1 position was partitioned between 1 ml of 0.05M NaOH and 5 ml of *n*-heptane. The organic layer was transferred to a tube containing 5 ml of 0.2N HCl. This aqueous layer was alkalinized with 6N NaOH and reextracted with 5 ml of $CHCl_3$. This was dried and reconstituted in 500 μl of ethanol. Assuming 100% purity for the initial methadone, the 1 μl of solution added will produce a concentration of 190 ng of methadone free base per 1 ml of plasma.

For detailed drug binding studies, various concentrations of unlabeled methadone HCl (Merck) were made up in ethanol and 20 μl of each solution was added to 1 ml of protein solution along with the 1 μl of labeled methadone. This provided methadone concentrations of between 2.7 μM and 140 μM. Where no extra methadone beyond the tritiated substance was to be added, 20 μl of ethanol was added to the sample. The methadone HCl was checked for purity with both high-pressure liquid chromatographic and gas chromatographic–mass spectrometric (GC/MS) methods. In neither case were any impurities detected. Other drugs for the competition studies were used as received from the manufacturers. They were dissolved in ethanol to provide a concentration of 27 μM when 20 μl was added to 1 ml of the plasma or AAG solutions.

Human AAG (G9885, Sigma) and human albumin (essentially fatty acid free, A1887, Sigma) were used without further purification. Polyacrylamide gel electrophoresis indicated >95% purity for the AAG after staining with anazolene sodium. By radial immunoassay, no residual albumin was detected in an AAG solution containing 1.2 gm/dl. Its limit of detection was <5 mg/dl. Similarly, when the AAG assay was used, no AAG was found in a 4-gm/dl albumin solution; there the detection limit was 1 mg/dl. For some of the experiments, a solution was prepared containing 120 mg/dl AAG in the same phosphate-buffered saline solution used as the dialysate. A 4-gm/dl albumin solution was prepared in much the same way.

A least-squares fit of the AAG/methadone binding data to the formula $r/C = N_1 \cdot K_1/(1 + K_1 \cdot C) + N_2 \cdot K_2 (1 + K_2 \cdot C)$ was performed with MLAB.[6] Here r is the moles of bound methadone per mole of AAG, C is the free methadone concentration, and N and K represent the numbers and affinities of the two classes of binding sites to AAG that were observed. To correct for the dilution effect of the 1

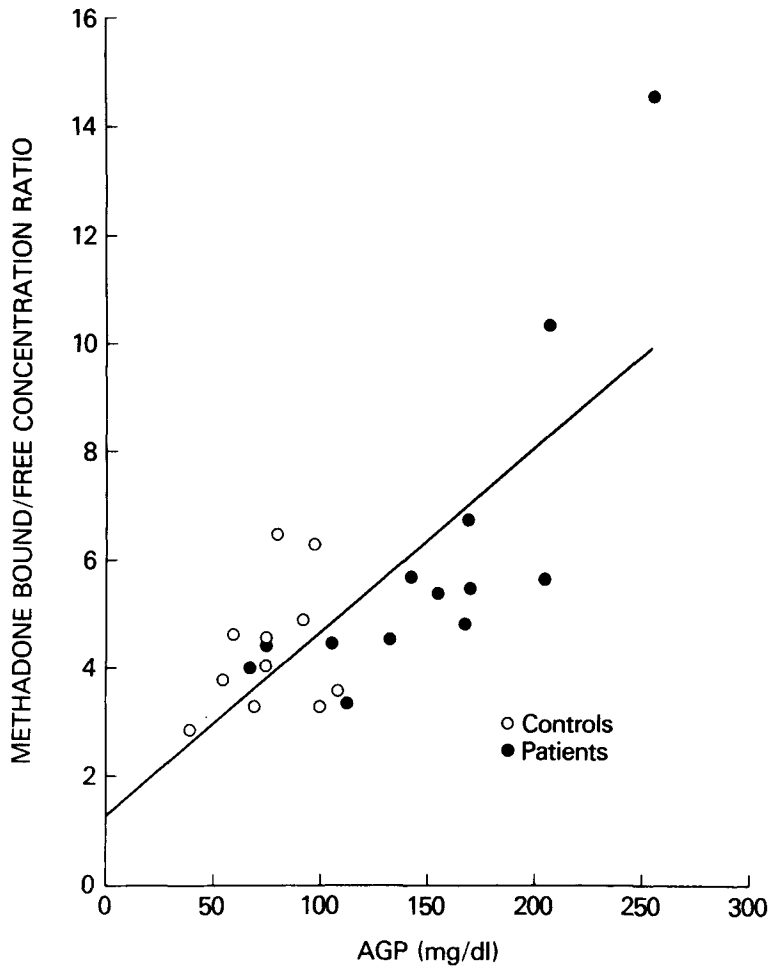

Fig. 1. Correlation plot of the bound/free concentration ratio for methadone and AAG. Normal subjects are represented by *open circles* and cancer patients are represented by *closed circles*. The regression equation is $B/F = 1.29 + 0.034 \times (AAG)$. The correlation coefficient $r = 0.76$.

ml of buffer when the binding fraction is low, the equation $C = T \cdot f_f/(1 + f_f)$ was used to calculate the free concentration, C, from the total amount added, T, and the observed free fraction, f_f. Linear regressions were performed on a Sharp EL-5100 calculator.

Results

We have reported that this group of cancer patients had significantly different amounts of AAG and albumin than the control group.[1] As in the earlier study in which propranolol was used, these patients also bind a significantly greater proportion of methadone than the controls. In contrast to the earlier study, this difference is smaller, the significance is reduced (Table I), and the correlation of methadone binding with AAG is weaker (Fig. 1). The intercept of the line in Fig. 1 implies 56% bound methadone in the absence of AAG. The slope gives an estimate of $N \cdot K_a$ for the methadone/AAG interaction of $1.5 \times 10^5 M^{-1}$, assuming only one class of binding site.

The result of an analysis of methadone binding to a pure solution of AAG is shown in Fig. 2. The data could be satisfactorily fit to a two-site model. The data were not sufficient to provide an accurate assessment of N_2 and K_2, although the computer-generated fit used values of 8.4 and $620 M^{-1}$. For the more avid site,

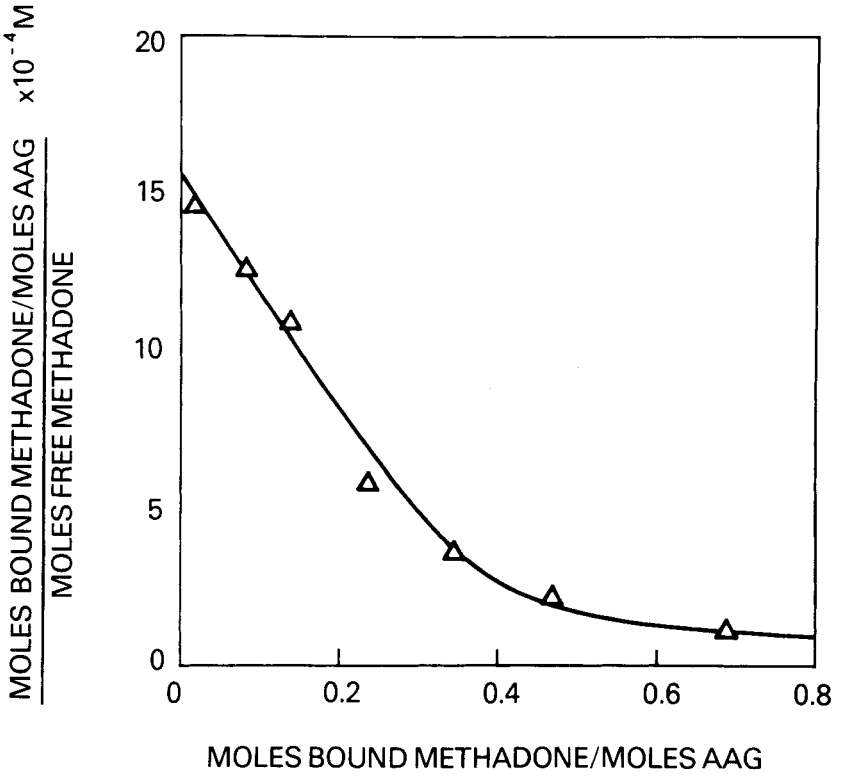

Fig. 2. Scatchard analysis of the interaction of methadone with AAG (2.7×10^{-5}M) at 37°.

the values were 0.38 and $4.0 \times 10^5 M^{-1}$, with standard errors of 0.08 and $0.8 \times 10^5 M^{-1}$. A parallel experiment, in which the AAG solution also contained 27 μM thioridazine, gave a one-site Scatchard with a good correlation coefficient ($r = 0.97$) and binding constants ($N = 0.30 \pm 0.04$ [mean ± SD] and $K_a = 4.7 \pm 0.8 \times 10^4 M^{-1}$).

Over the methadone concentration range of 3 to 54 μM, the bound percentage of methadone to a 4 gm/dl albumin solution was 15.8 ± 1.2% ($n = 8$). Since saturation did not occur, this number can be transformed into a value for $N \cdot K_a$ of $320 M^{-1}$.

Several other drugs known or suspected to bind to AAG were tested for inhibition of methadone binding, both with isolated AAG solutions and with human serum. The experimental data were generated in several ways. Because prochlorperazine and methadone might frequently be administered together to cancer patients, methadone binding to AAG was studied in solutions containing a series of concentrations of prochlorperazine from 0.03 to 300 μM. At 27 μM prochlorperazine the methadone bound/free concentration ratio was reduced 59%. This molarity was subsequently used in competition experiments with other drugs. In addition to prochlorperazine, propranolol, imipramine, chlorpromazine, and thioridazine were studied in triplicate with a 120 mg/dl AAG solution and these drugs, along with desmethylimipramine and diazepam, were examined once each using a normal subject's plasma, which contained 76 mg/dl AAG and 3.80 gm/dl albumin. These data are listed in Table II.

Discussion

The data in Table I show that free fraction of methadone in cancer patients differs from that in the control population. The correlation in Fig. 1 indicates that most of this difference is related to the concentration of AAG, which is elevated in many malignancies[4, 9] and in other disease states.[14] The average difference in free fraction between the patients and the controls

Table II. *Displacement of methadone from AAG and human plasma by lipophilic basic drugs*

Added drug (27 μM)	*Mean (±SEM) fraction bound in AAG solution*	*Mean (±SEM) fraction bound in plasma*
None	0.80 (n = 7) 0.01	0.80 (n = 4) 0.01
Prochlorperazine	0.62*	0.78 (n = 1)
Propranolol	0.68 (n = 3) 0.004	0.78 (n = 1)
Chlorpromazine	0.58 (n = 3) 0.006	0.76 (n = 1)
Imipramine	0.57 (n = 3) 0.009	0.74 (n = 1)
Thioridazine	0.23 (n = 3) 0.006	0.64 (n = 1)
Desmethylimipramine		0.78 (n = 1)
Diazepam		0.82 (n = 1)
Methadone	0.50*	0.63 (n = 1)

*From analysis of binding curves.

(−20%) is smaller than the difference observed in the earlier study using propranolol (−33%).[1] An alteration of binding of this magnitude is not likely of clinical significance. It is noteworthy that there is substantial unpredictability of the free fraction in these cancer patients. There was a nearly fourfold range of free fractions (0.064 to 0.23) among cancer patients, while in the control group the range was only twofold (0.13 to 0.26). Differences of this magnitude could be significant if a drug had a narrow therapeutic range.

The data on the inhibition of methadone binding to AAG indicate that each added drug can displace methadone. Prochlorperazine is the only one of the drugs, so far as we know, the binding of which had not been reported. Its ability to displace methadone suggests that its binding to AAG is of the same order as the other drugs tested.

In a similar set of experiments, but using competition of imipramine binding to AAG rather than methadone, Kornguth et al.[7] found that a variety of antipsychotic drugs, as well as diazepam and propranolol, could displace their test drug. Although more experiments should be done before a firm conclusion is reached, it appears from these data that most, if not all, basic lipophilic drugs that bind avidly to the AAG molecule are capable of displacing one another.

A detailed interpretation of the results regarding displacement of methadone by the other drugs is made difficult by lack of information on the binding constants for these drugs to AAG. Such data are required for an accurate calculation of the free inhibitor concentration and the competition for binding sites.

We are not the first to report more than one class of binding site for AAG. Brinkschulte and Breyer-Pfaff[3] reported two sites for the binding of perazine, amitriptyline, and nortriptyline to AAG, with the NK product of the more avid site 6 to 24 times that of the less avid site. Kornguth et al.[7] commented that at higher concentrations of imipramine (25 to 80 μM) their plots showed the presence of another binding site. Recently El-Gamel et al.[5] described both a high- and low-affinity site for dipyridamole binding to AAG. Our measurements of AAGs purity indicate that the second site in our experiments was not due to contaminants, an artifact analogous to that described by Lima and Salzer.[8]

It is gratifying to note that the NK product determined from Fig. 1 is identical to the N_1K_1 product described by Fig. 2, $1.5 \times 10^5 M^{-1}$. We wondered whether one or both sites could be inhibited by other drugs and therefore performed a second experiment, this time using an AAG solution containing 27 μM thioridazine. From a Scatchard analysis, the constants found were inconsistent with the description of methadone binding seen in Fig. 2. Although the fit in

Fig. 2 did not provide an accurate assessment of the parameters N_2 and K_2, when the constants obtained from the thioridazine experiments were substituted N_2 and K_2, the curve generated clearly did not fit the points properly. Therefore, the interaction of thioridazine with the binding sites for methadone is not simple and must be further studied if any conclusions are to be drawn about the mechanism of the displacement.

The important clinical question is not whether these drugs can interact, but rather, whether it is likely that they will interact? The K_a for methadone binding to its most important site, AAG, is $4 \times 10^5 M^{-1}$. Assuming 80% binding, this can be converted to a half-saturating methadone concentration of 4600 ng/ml. This is well above the plasma concentrations of methadone that have been reported in methadone-maintained addicts (100 to 200 ng/ml)[11, 15] who, in general, receive larger doses of methadone than do those taking it as an analgesic. Thus, saturation of methadone binding sites by conventional doses of methadone is unlikely.

It is also unlikely that a drug interaction would occur involving displacement from AAG. With the exception of thioridazine, the affinity constants for the drugs tested appear in the $10^5 M^{-1}$ range. Each of these interacting drugs is relatively potent inasmuch as their therapeutic ranges, where known, are all below 1 μg/ml.[2] All are highly bound and their low free concentrations will further lessen the probability of an interaction with AAG. Thioridazine has an extremely avid binding as assessed by equilibrium dialysis from patient's serum,[12] competitive inhibition of imipramine binding to AAG,[7] or in the present work as a displacer of methadone. Again, its low free fraction (0.0015)[12] suggests that free concentrations of thioridazine will also be well below effective displacing concentrations. Thus it is not likely that in subjects receiving customary doses of methadone and any of these other drugs the effect of methadone would be significantly enhanced by displacement from plasma proteins.

These considerations are further tempered by the fact that a substantial percentage of methadone binding is to substances other than AAG or albumin. A comparison of the first and second columns of Table II shows that plasma markedly attenuates the interaction produced by what are relatively high concentrations of the second drug on AAG binding. Romach et al.[16] also noted the residual binding of methadone to plasma components other than AAG and albumin and suggested that lipoproteins were responsible.

Our investigation has not uncovered any explanation for the adverse reaction experienced by the patient who was the origin of this research. Our findings show that there is substantial variation among cancer patients in the bound fraction of methadone. On that basis, narcotic analgesic drugs that are less avidly bound than methadone might be preferred because there would be one less source of possible variability. Olsen[13] reported that morphine was 35% bound to plasma proteins. In spite of this apparent advantage, Sawe et al.[17] found that the physiologic disposition of morphine was very variable in cancer patients, although they did not compare its disposition in another population. Given these descriptions of the variable kinetics of these drugs in cancer, a more quantitative approach to dosing narcotic analgesics, such as repetitive measurements of their free plasma concentrations, might result in improved efficacy and safety.

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