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Tianeptine binding to human plasma proteins and plasma from patients with hepatic cirrhosis or renal failure

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1 The binding of tianeptine to human plasma, isolated plasma proteins, red blood cells and to plasma from patients with cirrhosis or renal failure was studied *in vitro* by equilibrium dialysis.
2 Tianeptine is highly bound to plasma (95%) at therapeutic concentrations (0.3–1 μM). No saturation of the binding sites was seen.
3 Human serum albumin (HSA) was shown to be mainly responsible for this binding (94%) with a saturable process characterized by one binding site with a moderate affinity ($K_a = 4.2 \times 10^4$ M^{-1}) and a non-saturable process with a low total affinity ($nK_a = 1.2 \times 10^4$ M^{-1}).
4 Like many basic and amphoteric drugs, tianeptine showed a saturable binding to α_1-acid glycoprotein (AAG) with one site and a moderate affinity ($K_a = 3.7 \times 10^4$ M^{-1}). Its binding to lipoproteins and red blood cells (RBC) was weak and non-saturable. Over the range of therapeutic drug concentrations (0.3 − 1 μM), the unbound fraction in blood remains constant (4.5%).
5 Interactions were studied using non-esterified fatty acids (NEFA) at pathological concentrations; they altered tianeptine binding to plasma and to isolated HSA. Tianeptine seems to bind to a HSA site different from sites I (warfarin) and II (diazepam), but close to site II. It also shares the only basic-site on AAG. However, at therapeutic drug concentrations (0.3–1 μM), not all of these interactions occur.
6 The binding of tianeptine varied according to HSA, AAG and NEFA concentrations both in patients and healthy subjects. In patients with chronic renal failure having high NEFA concentrations the unbound fraction of tianeptine (f_u) increased from 0.045 to 0.153 compared with normal ($P < 0.001$). In cirrhotic patients, with relatively low HSA concentrations, the f_u of tianeptine increased from 0.045 to 0.088 compared with normal ($P < 0.01$).
7 Multiple regression analysis of all of the data indicated that the f_u of tianeptine was related significantly to HSA, NEFA and AAG concentrations, with a particularly strong correlation with NEFA concentrations. Therefore, variation of HSA and NEFA concentrations in patients on maintenance therapy may cause an increase of tianeptine f_u.

Keywords tianeptine α_1-acid glycoprotein human serum albumin protein binding elderly cirrhosis renal failure

Introduction

Tricyclic antidepressants have been shown to be bound extensively to plasma proteins (Braithwaite, 1980). Tianeptine (7-[(3-chloro-6, 11, dihydro-6-methyl-dibenzo [c,f] [1,2] thiazepin-11-yl) amino] heptanoic acid S-S dioxyde, sodium salt) is a new antidepressant agent (Defrance & Kamoun, 1988; Lôo *et al.*, 1987, 1988) with a mechanism of action quite different from that of tricyclic antidepressants since it was shown to increase the uptake of serotonin in brain and platelets (Baccino *et al.*, 1988; Kato & Weitsch, 1988; Mennini *et al.*, 1987). Its chemical structure is related to that of classical tricyclic antidepressants but differs by the presence of a sulphonamide function in the central nucleus and a lateral amino acid chain. Tianeptine is amphoteric with two pKa values, 4.4 (acidic) and 6.86 (basic), and is weakly lipophilic (log P at pH 7.4 = 1.06).

Tianeptine has been recently marketed in France in 1987 and will be marketed in other European countries soon. The aim of this study was to measure the binding of tianeptine to human plasma, isolated proteins and red blood cells (RBC). We have also compared the binding of tianeptine in plasma from healthy (young and elderly) and diseased subjects.

Methods

Drugs

Unlabelled tianeptine and [^{14}C]-tianeptine ([^{14}C]-S1574, 30 Ci mol^{-1}) were provided by the Institut de Recherches Internationales Servier, Neuilly, France. The radiochemical purity (99%) was determined by t.l.c. (silica gel F-254, MERCK) using chloroform/methanol as the mobile phase (90:10, v:v). Stock solutions of [^{14}C]-tianeptine were prepared in water and then diluted in phosphate buffer (0.067 M) to give concentrations from 0.3 to 400 μM (therapeutic range = 0.3–1 μM).

The possibility of inhibition of binding by warfarin (Merrell-Toraude), diazepam (Roche) and binedaline (Cassenne), was also studied.

Human plasma and isolated proteins

A pool of human plasma was obtained from drug-free healthy volunteers (total protein = 70.6 g l^{-1}; albumin (HSA) = 660 μM; α_1-acid glycoprotein (AAG) = 18 μM). HSA and AAG concentrations were measured using Beckman kits (663 000 and 662 996) and the Beckman Immunochemistry System ICS (Beckman Instruments) which is based on nephelometric measurement. Non-esterified fatty acid (NEFA) concentrations were measured by a colorimetric enzymatic method (NEFA C Kit number 46 551, Biolyon). The total concentration was 434 μM and the molar ratio NEFA/HSA was 0.657. The plasma pool was stored at −30° C.

HSA (Sigma A-1887) was dissolved in phosphate buffer, pH 7.4, at a concentration of 50 μM. The molar ratio of NEFA to HSA was 0.04 (Birkett *et al.*, 1978).

HSA containing 1000 μM NEFA (Sigma A-2386) was dissolved in phosphate buffer, pH 7.4, at a concentration of 660 μM. The molar ratio of NEFA to HSA was 1.51.

AAG (electrophoretic purity = 99%; Behringwerke) was also dissolved in phosphate buffer, pH 7.4, at a concentration of 20 μM. Lipoproteins (VLDL, LDL and HDL) were isolated by ultracentrifugation (Beckman L5-50B, rotor 50 Ti) from pooled normolipidaemic human serum, according to the procedure described by Glasson *et al.* (1982). The concentration of each lipoprotein was measured by the method of Lowry *et al.* (1951) and their molar concentrations were calculated using their average protein percentage and molecular weight: VLDL (10%, 10^7 Da), LDL (20%; 3.10^6 Da) and HDL (50%; 3.10^5 Da).

Red blood cells (RBC)

Blood was collected from a normal volunteer into heparinized tubes and centrifuged for 20 min at 1000 *g* at 4° C. The buffy coat and the plasma were separated. The RBCs were washed three times with 0.9% w/v NaCl and adjusted to a haematocrit of 47 in an isotonic saline glucose buffer (NaCl : 100 mM; KH_2PO_4 : 5 mM; Na_2HPO_4 : 20 mM; $CaCl_2$: 2.5 mM; $MgSO_4$: 1 mM; glucose : 5 mM, pH 7.4). In addition, the same haematocrit (47) was prepared with the pooled plasma and RBC. [^{14}C]-tianeptine in blood was incubated at 37° C for 15 min then the blood was centrifuged at 1000 *g* at 4° C for 15 min. [^{14}C]-tianeptine concentrations were measured in RBC and in supernatant (buffer or plasma). [^{14}C]-tianeptine concentrations in RBC (C_{RBC}) were calculated from:

$$C_{RBC} = \frac{C_{blood} - C_{plasma\ or\ buffer}\,(1 - H)}{H} \quad (1)$$

where H is the haematocrit value.

The fraction of tianeptine in RBC (f_{RBC}) was calculated from:

$$f_{RBC} = \frac{C_{RBC}}{C_{tot}} \times H \quad (2)$$

where C_{tot} was the total concentration of tianeptine. Assuming passive diffusion, the free fraction of tianeptine in RBC (fu_{RBC}) becomes:

$$fu_{RBC} = \frac{H}{1 + fb/fu\,(1 - H)} \quad (3)$$

where fu and fb represent the free and bound fractions of tianeptine in plasma, respectively.

Binding experiments

The binding of [^{14}C]-tianeptine to isolated proteins and to plasma was measured by equilibrium dialysis. Experiments were carried out at 37° C, pH 7.4 (phosphate buffer 0.067 M) for 3 h with constant stirring at 20 rev min^{-1} (Dianorm®) and without appreciable accumulation of fluid on the protein side of the dialysis compartment. The two compartments were separated by a semipermeable membrane (Visking, pore size 150 nm, cut off 12000 Da). No significant binding to the dialysis membrane was observed. The coefficient of variation of triplicate determinations was < 5%.

Subjects and sample collection

Plasma samples from 51 subjects were studied:
Control group (n = 12): Twelve men (21 to 27 years old) free from drugs and without clinical or biochemical signs of disease.
Elderly subjects (n = 12): Six men and six women (70 to 86 years old) with no signs of renal or hepatic insufficiency.
Patients with cirrhosis (n = 13): Nine men and four women (31 to 75 years old) with a clinical and biological diagnosis of hepatic cirrhosis, classification C (score between 10 and 15) according to the criteria of Child-Pugh (Pugh *et al.*, 1973).
Patients with chronic renal failure (n = 14): Seven men and seven women (20 to 85 years old) with clinical and biological evidence of renal failure (plasma creatinine : 1036 ± 222 μM (mean ± s.d.)). Samples from these patients were taken before haemodialysis.

Patients were not taking any drug known to be highly bound to AAG or HSA. Blood from the subjects who were all fasting was collected in heparinized tubes and centrifuged (10 min at 3000 rpm) to yield plasma, which was separated and frozen at −20° C until used. AAG, HSA and NEFA concentrations were measured as described above.

Calculation of binding parameters

At equilibrium, the concentrations in each compartment of the dialysis cell were measured by liquid scintillation counting (Packard Tricarb 460 CD). Free (F) and bound (B) molar concentrations of the drugs were calculated. The bound drug fraction (fb), expressed as a percentage, was calculated from

$$fb = \frac{B}{B + F} \times 100 \quad (4)$$

Values of B were plotted against F. In those cases where binding was found to be a saturable process, the binding parameters N (total concentration of binding sites), n (number of binding sites) and K_a (affinity constant) were calculated from:

$$B = \frac{NK_aF}{1 + K_aF} = \frac{nRK_aF}{1 + K_aF} \quad (5)$$

where R is the molar concentration of the protein. When the binding was non-saturable, B was related to F by equation (6):

$$B = NK_aF = nRK_aF \quad (6)$$

Where NK_a and nK_a represent the binding coefficient and the total affinity, respectively.

When a saturable and a non-saturable process were present together, B was related to F by equation (7):

$$B = \frac{N_1K_{a1}F}{1 + K_{a1}F} + N_2K_{a2}F = \frac{n_1R_1K_{a1}F}{1 + K_{a1}F} + n_2R_2K_{a2}F \quad (7)$$

where the indices 1 and 2 denote the first and second classes of binding sites, respectively.

All parameters were calculated using a non-linear least-squares regression method which employed a Gauss-Newton algorithm.

The plasma and blood binding of tianeptine was simulated at non-saturating concentrations. For each blood protein concentration studied, the total fraction (B/C_{tot}) of tianeptine bound to human plasma or blood was calculated from:

$$B/C_{tot} = \Sigma\, Bi/C_{tot} = \Sigma\, NiK_ai/(1 + \sum_{j=1}^{m} NjK_aj) \quad (8)$$

where Bi/C_{tot} denotes the fraction of tianeptine bound to one class of binding sites (i) on one type of protein (j), and $Nj K_a j$ is the binding coefficient of the protein.

Statistical analysis

All data were analysed with a uniform weighting of one. The binding percentages and parameters were expressed as means ± s.d. or means ± s.e. mean of 3–5 measurements.

Patients were divided into four groups for statistical analysis, healthy and elderly subjects, patients with hepatic cirrhosis and chronic renal failure. As the number of observations for each group was small, correspondence to a Gaussian distribution was tested with the Shapiro-Wilk test. When the distribution was non-Gaussian and the variances were very different, comparison of parameters between each group was made with the Kruskal-Wallis test.

An analysis was also done to determine whether the plasma binding of tianeptine could be described by a linear or a nonlinear model with uniform weighting of one. The free fraction of tianeptine (f_u) was calculated from:

$$f_u = \frac{1}{1 + nK_a \cdot HSA + nK_a \cdot AAG + \alpha \cdot NEFA} \quad (9)$$

provided that the dissociation constant (K_d) of the drug-protein complex was much higher than the free drug concentration.

nK_a, HSA, AAG, NEFA and α are the total affinity (product of the number of binding sites and the association constant), the HSA, AAG and NEFA concentrations in each patient and a correlation factor, respectively.

Results

[^{14}C]-Tianeptine binding to human plasma and isolated proteins

In the concentration range 0.3–1 μM, [^{14}C]-tianeptine was highly bound to plasma to a constant extent. The mean percentage binding was 95.27% ± 0.83 (mean ± s.d.) (Table 1). This binding was non-saturable with a binding coefficient of nK_a = 20.14 ± 0.17 (mean ± s.d.).

Over the same range of [^{14}C]-tianeptine concentrations, the percentage binding HSA (660 μM) also remained constant and was identical to that obtained with plasma (Table 1). However, the binding of [^{14}C]-tianeptine (0.5–400 μM) to 50 μM HSA involved both a saturable and a non-saturable process (Figure 1). A Scatchard plot was non-linear and a direct plot (B *vs* F) showed one class of saturable

Table 1 The percentage binding of [^{14}C]-tianeptine to human plasma and to individual plasma proteins (mean ± s.d.)

Proteins (μM)	*[^{14}C]-tianeptine concentration (μM)* 0.3	0.6	1
Plasma (HSA = 660) (AAG = 18)	95.31 ± 0.22	96.08 ± 0.28	94.42 ± 0.35
HSA (660)	95.25 ± 1.04	95.56 ± 0.31	95.80 ± 1.61
HSA (660) +NEFA (1000)	*92.74 ± 0.39	*92.38 ± 1.86	*93.21 ± 0.70
AAG (20)	33.54 ± 1.44	37.17 ± 1.86	34.96 ± 2.23
HDL (13.3)	39.87 ± 1.99	37.72 ± 1.87	36.35 ± 2.04
LDL (1.3)	30.26 ± 1.23	25.85 ± 1.63	29.98 ± 1.45
VLDL (0.15)	7.91 ± 0.57	6.72 ± 1.21	7.02 ± 2.05
γ-globulins (100)	3.88 ± 0.12	4.45 ± 0.07	7.36 ± 0.53

* $P < 0.01$ compared with HSA (660 μM).

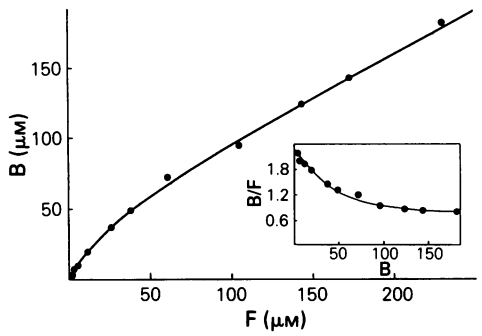

Figure 1 [^{14}C]-tianeptine binding to HSA (50 μM) studied by equilibrium dialysis at 37° C, pH 7.4, with 1 to 400 μM [^{14}C]-tianeptine.
Insert: Scatchard plot.

binding sites with n = 0.83 and $K_a = 4.2 \times 10^4$ M^{-1}, and one class of non-saturable sites with $nK_a = 1.2 \times 10^4$ M^{-1} (Table 2).

With HSA containing NEFA (1000 μM) the percentage binding of [^{14}C]-tianeptine (0.3–1 μM) to HSA (660 μM) decreased from 95.53 to 92.77%.

These results suggest that proteins other than HSA are responsible for the plasma binding of [^{14}C]-tianeptine since the plasma used contained 434 μM NEFA and the HSA preparation contained 2 μM NEFA.

Five other proteins were able to bind [^{14}C]-tianeptine, namely AAG, VLDL, LDL, HDL and γ-globulins. [^{14}C]-tianeptine binding to 20 μM AAG was saturable over the concentration range 0.5 to 100 μM. The binding involved one class of binding sites with n = 0.66 ± 0.10 and $K_a = 3.7 \pm 0.6 \times 10^4$ M^{-1} (mean ± s.d.) (Table 2). However, at therapeutic drug concentrations (0.3–1 μM) saturation was not observed (Table 1).

The binding of tianeptine to VLDL and γ-globulins did not exceed 8% (Table 1). In contrast the lipoproteins LDL and HDL bound 38% and 29% of [^{14}C]-tianeptine, respectively, at therapeutic drug concentrations (Table 1). When [^{14}C]-tianeptine was dialysed over a wide range of concentrations (0.5–300 μM for LDL; 0.5–1100 μM for HDL), its binding was non-saturable to both LDL and HDL with nK_a values of 25×10^4 and 2.1×10^4 M^{-1}, respectively (Table 2).

[^{14}C]-tianeptine binding to RBC:

When the concentration of [^{14}C]-tianeptine was varied from 0.3 to 1 μM, its binding remained constant at 72% (Table 3). In the presence of plasma, this binding decreased four-fold showing that tianeptine has a higher affinity for plasma proteins than for RBC. In addition, the

Table 2 Binding constants for the interaction of [^{14}C]-tianeptine with various plasma proteins (mean ± s.d.)

Proteins (μM)		n	$K_a \times 10^4$ (M^{-1})	$nK_a \times 10^4$ (M^{-1})
HSA (50)	first class	0.83 ± 0.21	4.2 ± 1.1	3.5 ± 1
	second class	—	—	1.2 ± 0.1
AAG (20)		0.66 ± 0.10	3.7 ± 0.6	2.4 ± 0.7
HDL (13.3)		—	—	2.1 ± 0.1
LDL (0.44)		—	—	25 ± 2

Table 3 [^{14}C]-tianeptine binding to RBC (mean ± s.d.)

	[^{14}C]-tianeptine (μM)		
	0.3	*0.6*	*1*
f_{RBC} (%)	72.6 ± 1.4	71.8 ± 0.8	72.5 ± 1.3
f_{RBC} (plasma) (%)	18.4 ± 1.2	18.4 ± 0.8	17.0 ± 0.7
fu_{RBC} (%)	3.2	3.2	3.2
f_{RBC} (plasma)/fu_{RBC}	5.8	5.8	5.3

f_{RBC} = tianeptine fraction in RBC in the presence of buffer.
f_{RBC} (plasma) = tianeptine fraction in RBC in the presence of plasma.
fu_{RBC} = the tianeptine free fraction in RBC.
The haematocrit was 47.

Table 4 Inhibition of [^{14}C]-tianeptine binding to HSA (mean ± s.d.) The concentration of [^{14}C]-tianeptine and HSA were 0.5 and 50 μM, respectively

Inhibitors (μM)	*[^{14}C]-tianeptine binding (%)*
0	67.4 ± 0.9
Warfarin	
500	45.4 ± 2.0*
1000	45.1 ± 6.2*
Diazepam	
500	46.8 ± 2.1*
1000	34.2 ± 2.7*
Tianeptine	
1000	23.6 ± 1.3*

* $P < 0.001$ compared with the binding of [^{14}C]-tianeptine alone.

Table 5 Inhibition of [^{14}C]-tianeptine binding to AAG (mean ± s.d.) The concentrations of [^{14}C]-tianeptine and AAG were 0.5 and 20 μM, respectively

Binedaline (μM)	*[^{14}C]-tianeptine binding (%)*
0	34.0 ± 1.6
0.5	31.7 ± 2.7*
1	24.0 ± 3.9**
5	11.8 ± 1.2**
10	6.5 ± 0.7**
20	2.7 ± 1.1**
50	1.0 ± 0.1**

* $P < 0.01$; ** $P < 0.001$ compared with the binding of [^{14}C]-tianeptine alone.

f_{RBC}/fu_{RBC} ratio of 5.3 to 5.8 indicated that tianeptine is bound either to RBC membranes, to haemoglobin, or is concentrated within the RBC (Table 3).

Inhibition of [^{14}C]-tianeptine binding to HSA

In order to identify the binding site of tianeptine on HSA, its binding was studied in the presence and absence of two markers of different sites, namely warfarin and diazepam. This was done at a therapeutic plasma concentration of tianeptine (0.5 μM) and an HSA concentration of 50 μM. At saturating concentrations of diazepam and warfarin (500 μM), both drugs lowered the binding of [^{14}C]-tianeptine from 67 to 46% (Table 4). When higher concentrations were used (1000 μM), diazepam caused a greater decrease of tianeptine binding but warfarin did not. However 1000 μM of warfarin, diazepam or tianeptine were unable to inhibit completely the binding of [^{14}C]-tianeptine (Table 4).

Inhibition of [^{14}C]-tianeptine binding to AAG

Table 5 shows that tianeptine bound to isolated AAG was displaced by low concentrations of binedaline, which is a selective ligand for the AAG binding site (Morin *et al.*, 1985).

This decrease in tianeptine binding was proportional to the concentration of binedaline; 50 μM binedaline displaced [^{14}C]-tianeptine completely from AAG.

[^{14}C]-tianeptine binding in plasma from healthy subjects and from patients with disease

The means ± s.e. mean and ranges of the variables measured in the different subjects are shown in Table 6. Differences in HSA and AAG concentrations ($P < 0.01$) were observed between the elderly and the healthy subjects. Differences in HSA concentrations and free tianeptine fractions (*fu*) were found in cirrhotic patients as compared with healthy subjects. Creatinine, AAG, NEFA concentrations and *fu* values of tianeptine were significantly higher in patients with chronic renal failure as compared with those of healthy subjects (Table 6).

Multiple regression analysis of all of the data indicated that the *fu* of tianeptine was related significantly to HSA, NEFA and AAG concentrations (Table 6). The variability in the *fu* of tianeptine was highly correlated (multiple $r = 0.436$) with HSA (25%) and NEFA (72%) concentrations and to a lesser extent with AAG concentration (3%). The partial regression coefficients gave estimates of nK_a for the interaction of tianeptine with HSA of 44 ± 4 mM^{-1} and with AAG of 143 ± 8 mM^{-1} (mean ± s.d.). The ordinate intercept was not significantly different from zero.

Discussion

In healthy volunteers, tianeptine has an average half-life of 2.5 h with an apparent volume of distribution and a total plasma clearance of 0.77 l kg^{-1} and 241 ml min^{-1}, respectively (Royer *et al.*, 1988). It is metabolized to [7-(3-chloro-6,11-dihydro-5, 5-dioxo-6-methyldi-

Table 6 Plasma concentrations of bilirubin, creatinine, HSA, AAG, NEFA and the free fraction of tianeptine (*fu*) (mean ± s.d.)

	Healthy subjects (n = *12*)	*Elderly subjects* (n = *12*)	*Cirrhotic patients* (n = *13*)	*Patients with chronic renal failure* (n = *14*)
Bilirubin (μM)	18.5 ± 1.1	11.8 ± 0.9	146.9 ± 46.5	5.3 ± 0.7**
Creatinine (μM)	91.0 ± 3.2	79.1 ± 5.6	84.2 ± 9.5	1036 ± 59**
HSA (μM)	691.7 ± 11.0	515.8 ± 19.5**	356.6 ± 16.6**	572 ± 23
AAG (μM)	11.41 ± 0.51	21.49 ± 1.77**	10.92 ± 2.36	17.68 ± 1.54*
NEFA (μM)	524 ± 77	619 ± 114	767 ± 95	3403 ± 277**
fu	0.045 ± 0.001	0.051 ± 0.002	0.088 ± 0.007**	0.153 ± 0.006**

* $P < 0.05$; ** $P < 0.01$ compared with healthy subjects.
The stepwise multivariate analysis of the relationship between the free fraction of tianeptine (*fu*) and biological variables (HSA, AAG and NEFA) gave values (± s.d.) of nK_a HSA, nK_a AAG and α of 0.044 ± 0.004 ($P < 0.01$), 0.143 ± 0.008 ($P < 0.01$) and (2.08 ± 0.20) 10^{-5} ($P < 0.01$), respectively. Student's *t*-test was used with d.f. of *t* value (51 points minus number of parameters). The multiple *r* was 0.436 and the uniform weighting was 1.

benzo (c,f) (1,2) thiazepin-11-ylamino) pentanoic acid] which is excreted by the kidney and metabolized in the liver (Bouyer *et al.*, 1988). Our results show that tianeptine is highly bound to plasma (≈ 95%) and that this binding is constant over the range of therapeutic drug concentrations (0.3–1 μM). Tianeptine is bound to HSA, AAG, LDL, HDL and RBC. Most of the binding is to HSA (94%), other proteins representing 3% of total binding (Table 7). The free percentage of tianeptine is about 3% and 4.5% in plasma and blood respectively. Our simulations suggest that the binding of tianeptine in blood is close to that in plasma, because RBC show a very low affinity for tianeptine compared to that of HSA. This heterogeneous binding explains the observation that the plasma binding percentage remains constant over the range of therapeutic drug concentrations (Table 1).

Table 7 Simulation of tianeptine binding in plasma and blood. The values were calculated for the therapeutic range of concentration of tianeptine (0.3–1 μM), which is below a saturating concentration, with normal plasma or blood.

Proteins	(μM)	nK_a	B/C_{tot} *Plasma*	*Blood*
HSA	670	34.80	93.80	87.00
AAG	20	0.74	2.00	1.85
LDL	1	0.25	0.67	0.62
HDL	15	0.31	0.84	0.77
RBC	H = 47	1.16	—	5.27
Plasma		36.10	97.31	—
Blood		21.01	—	95.51
fu_p			2.69	—
fu_b			—	4.49

H is the haematocrit (47) and fu_p and fu_b are the plasma and blood free fractions of tianeptine, respectively.

The binding of tianeptine to HSA (50 μM) was biphasic, with a saturable component described by a moderate affinity constant (4.2×10^4 M^{-1}) and a non-saturable component with a low nK_a value (1.2×10^4 M^{-1}). This finding is surprising because tianeptine is an amphoteric molecule and, as a rule, amphoteric and basic compounds, like imipramine (Tillement *et al.*, 1974), show non-saturable binding to HSA. At therapeutic plasma concentrations, tianeptine occupies about 0.2% of HSA binding sites.

The binding of tianeptine to AAG is also saturable with a similar affinity constant (3.7×10^4 M^{-1}). At 1 μM, tianeptine occupies about 2% of the AAG binding sites in plasma.

Tianeptine binds to one saturable binding site on AAG which seems to be shared by other basic drugs (Routledge, 1986) such as binedaline (Table 5 and Morin *et al.*, 1985), neuroleptics (Muller & Stillbauer, 1983), disopyramide (David *et al.*, 1983) and propisomide (Zini *et al.*, 1985).

The non-saturable binding of tianeptine to lipoproteins represents 1% of the overall plasma binding and may be regarded as a partition process between the saturable proteins (HSA and AAG) and lipoproteins. Thus tianeptine may be dissolved in the lipid part of lipoproteins (Glasson *et al.*, 1982). No significant binding to VLDL and γ-globulins was observed (Table 1).

The binding of tianeptine to RBC in buffer solutions remains constant (≈ 72%) within the range of therapeutic drug concentrations, while binding to RBC in plasma is also constant but

much lower (≈ 18%). If tianeptine diffuses passively into RBC, its fu_{RBC} value should be 0.032. Since the observed value was 0.18, this indicates that tianeptine binds to the RBC membrane in common with many other lipophilic drugs (Essassi *et al.*, 1987; Hamberger *et al.*, 1986).

NEFA (1000 μM) decreased tianeptine binding in the *in vitro* experiments (Table 1). However, several reports indicate that fatty acids attach to high affinity binding sites on HSA, different from those shared by anionic and cationic drugs (King, 1973; Koh & Means, 1979; Peters, 1977).

The displacement of bound tianeptine from HSA may, therefore, be explained by an allosteric effect induced by NEFA binding to their separate sites.

Anionic drugs appear to bind to at least two binding sites on HSA, namely site I (warfarin site) and site II (diazepam site) (Sjoholm *et al.*, 1979; Sudlow *et al.*, 1976), although this suggestion has been challenged (Brodersen *et al.*, 1984; Honore & Brodersen 1984; Iwatsuru *et al.*, 1982; Kragh-Hansen, 1985; Maruyama *et al.*, 1984). Our results show that the binding of tianeptine can be decreased but not fully inhibited by high concentrations of diazepam or warfarin (Table 4). However, diazepam displaces tianeptine more strongly than warfarin, but much less than unlabelled tianeptine. Therefore, the binding site of tianeptine could be different from sites I and II, but near to the diazepam site. Thus competitive or non-competitive inhibition could occur between tianeptine and drugs sharing the diazepam site, such as benzodiazepines and aromatic carboxylic acids, because these latter drugs have association constants higher than those of tianeptine. Moreover, these drugs have therapeutic plasma concentrations similar to those of tianeptine (≈ 1 μM) and will not occupy all of the available sites on HSA. Even if tianeptine was displaced by another drug from HSA, which is unlikely, this displacement would be minimized or offset by the 'buffering' effect of binding sites on other proteins. Tianeptine would then bind to the non-saturable sites of HSA, lipoproteins, RBC and/or to the saturable site of AAG.

Previous studies have shown that albumin, NEFA and AAG concentrations are, respectively, the major determinants of the binding of acidic and basic drugs (Piafsky, 1980; Pritchard *et al.*, 1983). Our data show that the plasma binding of tianeptine in patients with chronic renal failure was significantly lower than in the other three groups. This decrease may be ascribed to the higher NEFA concentration in those subjects in spite of a net increase in AAG concentration (Table 6) (Baruzzi *et al.*, 1986; Pedersen *et al.*, 1987). Acute renal failure is known to increase NEFA concentrations (Tillement *et al.*, 1978) and NEFA have an affinity for albumin ($K_a = 10^7$–10^8 M^{-1}) greater than that of many drugs and so can inhibit their binding to albumin (Rudman *et al.*, 1971). This is so for acidic drugs (Bowmer & Lindup, 1982) and also for basic drugs (Brown *et al.*, 1981; Horiuchi *et al.*, 1989; Kessler *et al.*, 1979).

As a general rule, patients with chronic renal failure do not show increased NEFA concentrations. Our patients were free of drug medication and had been fasted 1 h prior to haemodialysis. They received heparin (4000 Units) just before haemodialysis. The high level of NEFA observed may be due to the heparin injection resulting in the release of free fatty acids from triglycerides by activated lipoprotein lipase.

The increase in the *fu* value of tianeptine in cirrhotic patients could be accounted for by the decrease in HSA concentration (Tillement *et al.*, 1978). The major mechanism for this reduced binding seems to be impaired protein synthesis and/or the presence of endogenous binding inhibitors (Klotz *et al.* 1976; Thiessen *et al.*, 1976). Our findings may be explained by the significantly lower concentration of HSA because AAG concentrations were normal in the plasma of these patients in contrast to low values of AAG generally observed in patients with cirrhosis (Barré *et al.*, 1984; Hiramatsu *et al.*, 1976; Pedersen *et al.*, 1987).

In elderly subjects, the *fu* value of tianeptine did not differ from that observed in healthy subjects, in spite of a decrease in HSA concentration which was compensated by a large increase of AAG concentration (Table 6).

Our elderly subjects were in-patients and their elevated AAG concentrations might have been due to underlying inflammatory problems and/or rheumatism (Verbeek *et al.*, 1984).

In conclusion, our results show an increase in the free fraction of tianeptine in patients with chronic renal failure. This elevation of free fraction of tianeptine is less in cirrhotic patients and does not occur in patients with moderate liver cirrhosis (Royer *et al.*, 1989). Pharmacokinetic consequences of such a phenomenon relate essentially to the volume of distribution and the clearance. One can expect an increase in the volume of distribution more or less proportional to the increase in the plasma free fraction of tianeptine. With a highly cleared drug an increase in its *fu* may result in a concomitant increase of its free concentration leading to an increased pharmacological effect. With a poorly cleared drug, such as tianeptine, an increase in

its *fu* leads to an increased clearance and so a constant free drug concentration and thus an unchanged drug effect.

Although it is not possible to conclude on the basis of these results alone that adjustments of dosage are necessary, our observations may have clinical relevance since they agree with those of Royer *et al.* (1988), who recommended a decrease in dosage in patients with severe renal failure.

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