Disopyramide protein binding in plasma from patients with nephrotic syndrome during the exacerbation and remission phases

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1 Plasma protein binding of disopyramide was determined twice in plasma from seven patients with severe nephrotic syndrome when the disease activity was markedly different for each patient (mean $\pm$ s.d. of serum albumin 21 ± 4 vs 29 ± 3 g l⁻¹ ($P < 0.05$), proteinuria 13.6 ± 9.6 vs 2.2 ± 1.7 g day⁻¹ ($P < 0.01$), and α_1 -acid glycoprotein 0.34 ± 0.12 vs 0.95 ± 0.28 g l⁻¹ ($P < 0.01$) during the exacerbation vs remission phases, respectively).

2 Plasma samples containing disopyramide at the concentrations of 0.2–12.0 μ g ml⁻¹ were analysed by ultrafiltration. The free fractions at the proposed therapeutic concentration range (2.0–6.0 μ g ml⁻¹) were significantly ($P < 0.01$) greater during the exacerbation phase than during the remission phase.

3 Multiple linear regression analysis revealed that the free fraction of disopyramide at 3.0 μ g ml⁻¹ correlated much better with α_1 -acid glycoprotein (partial correlation coefficient = 0.85, $P < 0.01$) than with albumin (partial correlation coefficient = 0.25, $P < 0.05$).

4 The binding data of disopyramide analysed by a model assuming one specific binding site and nonspecific binding(s) demonstrated that the capacity constant correlated significantly ($r = 0.97$, $P < 0.001$) with plasma α_1 -acid glycoprotein.

5 The results suggest that a total concentration of disopyramide within the therapeutic range may not be a reliable guide for a safe dosing scheme in patients with severe nephrotic syndrome, particularly during the exacerbation period. It is recommended that free fraction of disopyramide and/or α_1 -acid glycoprotein concentration should be frequently monitored in those patients with the changing disease activity to whom this antiarrhythmic is to be prescribed.

Keywords disopyramide protein binding nephrotic syndrome change in clinical status α_1 -acid glycoprotein therapeutic drug monitoring

Introduction

Acidic drugs bind largely to albumin (Koch-Weser & Sellers, 1976). In contrast, the plasma protein binding of basic drugs differs from that of the more completely studied acidic drugs. α_1 -Acid glycoprotein (AAG) and albumin appear

to play an important role in the binding of these drugs (Piafsky, 1980; Routledge, 1986). AAG is known to show a large fluctuation due both to physiological and pathological conditions (Piafsky, 1980; Routledge, 1986). Associated

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with the change in plasma AAG concentrations, both increases and decreases in plasma protein binding of basic drugs have been reported (Borgå *et al.*, 1977; Piafsky *et al.*, 1978; Piafsky & Borgå, 1977; Echizen *et al.*, 1986).

Plasma protein binding of acidic drugs has been demonstrated to be grossly reduced in patients with nephrotic syndrome due to hypoalbuminaemia (Gugler & Azarnoff, 1977). However, there seems to be a paucity of knowledge regarding the protein binding of basic drugs in nephrotic syndrome (Gugler & Azarnoff, 1976; Tillement *et al.*, 1978; Piafsky, 1980). We have recently shown that the protein binding of a basic drug, disopyramide, in plasma from clinically stable or well-controlled nephrotic patients was not significantly different from that in the normal healthy controls (Echizen *et al.*, 1986) despite the fact that the serum albumin levels in these nephrotic patients (mean $\pm$ s.d., 26 ± 5 g l⁻¹) were substantially lower than those in the controls (45 ± 4 g l⁻¹). To our surprise, however, when we examined the protein binding of disopyramide twice within the same patients with severe nephrotic syndrome when their clinical status differed markedly, the disopyramide binding measured during the exacerbation phase of the disease was markedly reduced as compared with that measured during the remission or well-controlled phase. We report, herein, the intraindividual change in plasma protein binding of disopyramide associated with the change in disease activity in seven patients with severe nephrotic syndrome. One of the patients repeated each of the exacerbation and remission phases during the present study period, which gave us an opportunity to determine if the intraindividual change in disopyramide binding associated with the change in clinical status would reproducibly occur.

Methods

We investigated the *in vitro* plasma protein binding of disopyramide in plasma samples from seven patients with severe nephrotic syndrome during the exacerbation and remission phases. In all patients except for case 3 the protein binding of disopyramide was studied first during the exacerbation phase and then during the remission phase. In case 5 we could measure twice the changes in protein binding of disopyramide associated with the changes in the disease activity, since the patient repeated each of the phases during the present study period. The diagnosis of nephrotic syndrome was made on the basis of the clinical manifestations and laboratory find-

ings including proteinuria (> 3.0 g day⁻¹) and hypoalbuminaemia (< 30 g l⁻¹). Renal biopsy was done in all patients. The individual demographic characteristics and laboratory data pertinent to the present study are summarized in Table 1. All participants were thoroughly informed of the purpose of the study.

The concurrently administered drug(s) in each patient (Table 1) during the exacerbation and remission phases was held constant when this study was made, since plasma samples for disopyramide protein binding during these two phases were obtained before the initiation of and after the completion of the intensive treatment with the larger doses of corticosteroid (prednisolone or methylprednisolone) for exacerbated disease activity of nephrotic syndrome. In addition, plasma samples were obtained before the morning dose of concurrent drug(s) in each clinical status.

The detailed procedures for disopyramide plasma protein binding measurement were reported elsewhere (Echizen *et al.*, 1986). An all-glass system was used to obviate any possible artefact resulting from the blood collection and assay procedures. Plasma was separated by centrifugation at 3000 rev min⁻¹ for 10 min and stored at -70° C until analysed. Aliquots of plasma (800 μ l) to which disopyramide phosphate (Roussel Medica Co., Tokyo, Japan) had been added to achieve concentrations of 0.2, 1.0, 2.0, 3.0, 6.0, 8.0, 10.0, 12.0 μ g ml⁻¹ of disopyramide (to convert to SI units: 1 μ g ml⁻¹ = 2.95 μ mol l⁻¹) were analysed by ultrafiltration by using a Centrifree (Amicon Co., Danvers, USA) equipped with a YMT membrane (Amicon). The ultrafiltration device was centrifuged in an angled rotor at 2000 g for 5 min at 25 $^{\circ}$ C and 101 ± 4 μ l (mean $\pm$ s.d., $n = 6$) of ultrafiltrate was obtained. In preliminary experiments, the absence of nonspecific drug binding to the ultrafiltration unit and/or to the membrane was confirmed by the identity of drug concentrations measured in buffer solution and in ultrafiltrates at 0.2, 0.5, 1.0, 2.5, and 5.0 μ g ml⁻¹, respectively. In addition, the free fraction of disopyramide at 2.0 μ g ml⁻¹ measured by the current system was not sensitive to the changes in pH (7.0 to 8.0), filtration volume (100–200 μ l), and temperature (25 to 37 $^{\circ}$ C) as has been previously reported (Lima *et al.*, 1981; Norris *et al.*, 1984; Echizen *et al.*, 1986). Heparin *per se* had no effects on disopyramide protein binding as has been previously reported (Haughey & Lima, 1982; Echizen *et al.*, 1986). A fluorescence polarization immunoassay system (TDx System, Abbott Diagnostics, South Pasadena, USA) was used to measure total as well as free disopyramide con-

Table 1 Clinical characteristics and laboratory data of patients with nephrotic syndrome during exacerbation and remission phases

Case number	Age (years)	Sex	Clinical status	Body weight (kg)	Albumin (35-45 g l ⁻¹)	α_1 -Acid glycoprotein (0.5-1.0 g l ⁻¹)	Cholesterol (3.1-5.95 mmol l ⁻¹)	Proteinuria (g day ⁻¹)	Biopsy finding	Concurrent drug(s)
1	45	M	exacerbation phase	96.4	18	0.09	15.77	9.0	Focal glomerulosclerosis	Prednisolone, Aluminum hydroxide
			remission phase	62.5	26	1.1	7.71	3.0		
2	34	F	exacerbation phase	60.5	20	0.46	15.83	6.9	Minimal change	Prednisolone, Aluminum hydroxide
			remission phase	50.8	26	1.40	14.38	not detected		
3	37	F	exacerbation phase	49.3	26	0.38	15.31	10.0	Membranous nephropathy	Ketotifen fumarate
			remission phase	46.2	30	0.86	14.53	4.0		
4	26	F	exacerbation phase	68.0	20	0.38	8.87	14.0	Focal glomerulosclerosis	Prednisolone, Dipyridamole, Famotidine
			remission phase	57.5	32	1.20	8.43	1.4		
5*	20	M	exacerbation phase	68.0	16	0.44	15.21	34.0		Prednisolone, Dipyridamole, Aluminum hydroxide
			remission phase	51.0	35	0.70	7.35	2.0	Minimal change	Prednisolone, Dipyridamole, Famotidine
			exacerbation phase	55.8	30	0.40	9.46	12.0		
			remission phase	53.4	36	0.70	7.01	not detected		
6	21	M	exacerbation phase	64.0	21	0.30	14.43	6.0	Minimal change	Prednisolone
			remission phase	58.0	27	0.70	10.96	0.4		
7	48	F	exacerbation phase	57.5	28	0.36	12.15	15.0	Focal glomerulosclerosis	Famotidine, Aluminum hydroxide
			remission phase	55.0	29	0.70	9.34	4.5		

Normal values (Department of Clinical Chemistry, National Medical Center, Tokyo) are given in brackets.
 * Case 5 had repeated phases of exacerbation and remission of the nephrotic syndrome during the study period.

centrations in plasma and in ultrafiltrate as previously described (Echizen *et al.*, 1986). Within- and between-run coefficients of variation for measurement of drug concentrations at 0.15, 0.30, 0.60, 1.5, 3.0, 6.0 $\mu\text{g ml}^{-1}$ were all less than 5%. A recent study performed in our laboratory (Wang *et al.*, 1986) has demonstrated a good agreement between plasma disopyramide concentrations determined by the fluorescence polarization immunoassay and a high-performance liquid chromatography method within the concentration ranges reported herein. The free fraction of disopyramide was determined as the ratio of disopyramide concentration in ultrafiltrate to that in plasma. Repetitive measurements of the bound fractions of disopyramide at 2.0 $\mu\text{g ml}^{-1}$ gave a coefficient of variation of 2.7% ($n = 6$).

The binding of disopyramide was fitted to the following model assuming one specific binding site and nonspecific binding(s) (David *et al.*, 1980): $C_b = (B_{\max} \cdot K_a \cdot C_f) / (1 + K_a \cdot C_f) + NS \cdot C_f$, where C_b is the bound drug concentration, $B_{\max}$ is the capacity constant, K_a is the association constant for specific binding site, C_f is the unbound or free drug concentration, and NS is a nonspecific binding parameter. All of the parameters were estimated by nonlinear least-squares regression (Metzler, 1969). The contribution of the nonspecific binding to the total disopyramide binding at 3.0 $\mu\text{g ml}^{-1}$ during the exacerbation and remission phases was estimated by using the obtained binding parameters and an equation, $NS \cdot C_f / C_b$, in each patient.

AAG was measured by radial immunodiffusion on M-partigen plates (Behringwerk, Marburg, West Germany) using Protein Standard Serum (Behringwerk) as the quantitative standard. The coefficient of variation of this method at 0.92 g l⁻¹ was 5.5% ($n = 10$). All other laboratory data were obtained with an Auto-Analyzer (SMAC I, Technicon, Tarrytown, NY, USA) in the Department of Clinical Chemistry, National Medical Center, Tokyo.

Comparisons between the mean values obtained during the exacerbation and remission phases were made by using the paired Student's *t*-test. Multiple comparisons between the mean values were performed by ANOVA with the least significant test. To determine if either AAG or albumin level was more important in the protein binding of disopyramide within the therapeutic range, we examined the contribution of AAG and albumin levels to the overall variability in the protein binding of disopyramide at a near-midpoint of the therapeutic range, 3.0 $\mu\text{g ml}^{-1}$, by using simple and multiple linear regression analyses. Data are given as the mean

$\pm$ s.d. throughout the text. A P value less than 0.05 was considered statistically significant.

Results

The clinical status or disease activity of nephrotic syndrome significantly influenced the individual (Table 1) and the mean ($\pm$ s.d.) body weight and laboratory data (Figure 1). The mean $\pm$ s.d. values for body weight (66.2 ± 14.8 kg), serum albumin (21 ± 4 g l⁻¹), plasma AAG (0.34 ± 0.12 g l⁻¹), total serum cholesterol (13.9 ± 2.6 nmol l⁻¹), and proteinuria (13.6 ± 9.6 g day⁻¹) during the exacerbation phase were significantly ($P < 0.05$ to 0.01) different from the respective values (54.5 ± 5.5 kg; 29.3 ± 3.4 g l⁻¹; 0.95 ± 0.28 g l⁻¹; 10.4 ± 3.0 nmol l⁻¹ and 2.2 ± 1.7 g day⁻¹) during the remission phase (Figure 1). Proteinuria was not detected during the remission phase in case 2 and 5b (Table 1) with spectrophotometric assay in the Department of Clinical Chemistry, National Medical Center, Tokyo. Therefore, the overall mean $\pm$ s.d. of proteinuria was calculated by incorporating the value of urinary excretion of protein, zero g day⁻¹, for case 2 and 5b. The disease activity of nephrotic syndrome appears to have a greater influence on AAG than on albumin levels (Figure 1): the mean AAG and albumin levels measured during the remission phase were 2.5 and 1.3 times greater than those measured during the exacerbation phase, respectively.

Figure 2 illustrates the individual data on the plasma free fractions of disopyramide at the total disopyramide concentrations ranging from 0.2 to 12.0 μ g ml⁻¹ measured during the exacerbation and remission phases in our seven patients. The free fractions of disopyramide at the proposed therapeutic range, 2.0–6.0 μ g ml⁻¹ (Koch-Weser, 1979), measured during the exacer-

bation phase were greater than during the remission phase in all patients. The respective mean free fractions during the exacerbation phase (48 ± 13 , 57 ± 10 , and $70 \pm 6\%$) at 2.0, 3.0 and 6.0 μ g ml⁻¹ of the total disopyramide concentration were significantly ($P < 0.01$) greater than those during the remission phase (19 ± 6 , 28 ± 8 , and $44 \pm 10\%$). As can be seen in one patient (case 5a & b, Figure 2), the changes in disopyramide protein binding associated with the changes in clinical status were reproducible, although the total disopyramide concentration vs the free fraction relationships differed to certain extent between the two different disease activity phases. The overall mean $\pm$ s.d. values were fairly similar irrespective of which of the two data sets from case 5 were included. Therefore, all mean values reported in the paper used the data observed during the first exacerbation and remission sequence (case 5a, Figure 2).

Simple linear regression analysis revealed that the free fraction at the total disopyramide concentration of 3.0 μ g ml⁻¹ correlated better with AAG ($r = -0.93$, $P < 0.001$, Figure 3a) than with albumin ($r = -0.61$, $P < 0.05$, Figure 3b). Multiple linear regression analysis revealed that the contributing power of AAG to the overall variability in plasma protein binding of disopyramide at the total concentration of 3.0 μ g ml⁻¹ was much greater than that of albumin: the partial correlation coefficients of AAG and albumin were 0.82 ($P < 0.01$) and 0.25 ($P < 0.05$), respectively.

All binding data were well fitted to the proposed binding model. The average binding data observed during the exacerbation and remission phases are listed in Table 2. The mean capacity and association constants obtained from the patients during the remission phase as given in Table 2 were fairly similar to those obtained from the patients with well-controlled nephrotic

Figure 1 The mean $\pm$ s.d. values for body weight, serum albumin, plasma AAG, total serum cholesterol, and proteinuria observed during the exacerbation (▨) and remission (□) phases in seven patients with nephrotic syndrome. * $P < 0.05$ and ** $P < 0.01$ compared with the remission phase.

Figure 2 Plasma free fractions of disopyramide at the total concentrations from 0.2–12.0 $\mu\text{g ml}^{-1}$ (to convert to SI units: $1 \mu\text{g ml}^{-1} = 2.95 \mu\text{mol l}^{-1}$) measured during the exacerbation and remission phases of nephrotic syndrome in seven patients.

One patient (case 5) repeated each of the exacerbation and remission phases during the present study: **a** and **b** indicate the first and second courses of the exacerbation and remission phases, respectively. In case 2 no data were available for the protein binding of disopyramide at 0.2 $\mu\text{g ml}^{-1}$ due to scarcity of plasma samples.

The case numbers are the same as given in Table 1 where the individual demographic and laboratory data obtained during the exacerbation and remission phases are listed. ● = data obtained during the exacerbation phase; ○ = data obtained during the remission phase.

syndrome and from the healthy controls in our previous study (Echizen *et al.*, 1986). The disease activity of nephrotic syndrome had a significant effect on the capacity constant but not on the association constant: the mean capacity constant

observed during the exacerbation phase was significantly ($P < 0.01$) smaller than during the remission phase, whereas no significant differences were observed in the mean association constants observed during the two phases (Table 2).

Figure 3 Plasma free fractions of disopyramide at the total plasma concentration of $3.0 \mu\text{g ml}^{-1}$ in relation to plasma AAG and serum albumin.

The least-squares regression lines are: $y = -49.24x + 72.82$ ($r = -0.93$, $P < 0.001$) in **a** and $y = -1.76x + 88.21$ ($r = -0.61$, $P < 0.05$) in **b**. Both data obtained during the first and second phases of exacerbation and remission in case 5 (Table 1) are included. When either of the matched data during the repeated phases was used, the regression lines and the r values were fairly similar to and the significant level did not differ from those as given above. ● = data obtained during the exacerbation phase, ○ = data obtained during the remission phase.

Table 2 Protein binding parameters in patients with nephrotic syndrome during the exacerbation and remission phase.

	Capacity constant ($\times 10^{-6} \text{ mol l}^{-1}$)	Association constant ($\times 10^6 \text{ l mol}^{-1}$)	Nonspecific binding
Exacerbation phase	$3.23 \pm 1.12^{**}$	1.58 ± 0.45	$0.17 \pm 0.05^{*}$
Remission phase	7.82 ± 2.14	1.28 ± 0.42	0.39 ± 0.11

Data are the mean $\pm$ s.d.

* $P < 0.05$ and ** $P < 0.01$ compared with the remission phase.

The capacity constants correlated significantly ($r = 0.97$, $P < 0.001$) with plasma AAG concentrations (Figure 4). In addition, the nonspecific binding parameter observed during the remission phase was significantly ($P < 0.05$) greater than during the exacerbation phase (Table 2). Nonspecific binding parameter had no significant correlation with albumin levels ($r = 0.31$).

Discussion

There have been few investigations of intra-individual changes in protein binding of drugs during the changing clinical status of a disease. To our knowledge, this is the first report to demonstrate that the protein binding of a basic drug, disopyramide, is markedly reduced during

the exacerbation phase of nephrotic syndrome as compared with that during the remission phase. A similar change in the protein binding of other basic drugs might be expected to occur during the different clinical stages of nephrotic syndrome.

The mean free fractions of disopyramide within the proposed therapeutic range, $2.0\text{--}6.0 \mu\text{g ml}^{-1}$ (Koch-Weser, 1979), observed during the exacerbation phase were approximately twice those observed during the remission phase. Previous animal (Huang & Øie, 1982) and human studies (Thibonnier *et al.*, 1984; Whiting *et al.*, 1980) have shown that unbound disopyramide concentrations correlated better with the negative inotropic and ECG effects (i.e., prolongation of QT and QRS intervals) than the total disopyramide concentrations. Therefore, our results

Figure 4 Relationship between plasma AAG concentrations and capacity constants. The least-squares regression line is: $y = 8.35x + 0.427$ ($r = 0.97$, $P < 0.001$). Both data obtained during the first and second phases of exacerbation and remission in case 5 (Table 1) are included. When either of the two data sets from case 5 were included, the regression lines and the r -value were fairly similar to and the significant level did not differ from those as given above. ● = data obtained during the exacerbation phase, ○ = data obtained during the remission phase.

may imply that the pharmacodynamic response(s) to disopyramide would be augmented at a given total therapeutic concentration when this antiarrhythmic agent is administered to patients with severe nephrotic syndrome during the exacerbation phase, and that achieving a total disopyramide concentration within the therapeutic range ($2\text{--}6\text{ }\mu\text{g ml}^{-1}$) should not always be a reliable guide for the safe dosing scheme in these patients.

It has been shown that AAG rather than albumin is responsible for the protein binding of disopyramide (Lima *et al.*, 1981; Norris *et al.*, 1984; Echizen *et al.*, 1986). The multiple linear regression analysis on the disopyramide binding data obtained during the exacerbation and remission phases showed that the contributing power of AAG to the overall variability of the disopyramide protein binding at a disopyramide concentration of $3.0\text{ }\mu\text{g ml}^{-1}$ was much greater than that of albumin. In addition, the capacity constant for the specific binding site obtained

from our nephrotic patients during the exacerbation and remission phases correlated highly significantly with the plasma AAG level (Figure 4), whereas the mean association constant remained unchanged regardless of the disease activity. These results would support the idea that plasma AAG is a major binding protein for disopyramide within the therapeutic range. In this context, we recommend that AAG levels should be frequently monitored in severe nephrotic patients with rapidly changing clinical status to whom disopyramide is to be prescribed. This consideration might be valid for other basic drugs of which the major binding protein is AAG (Piafsky, 1980; Routledge, 1986).

To our surprise, the mean plasma AAG level in our patients with severe nephrotic syndrome showed a significant ($P < 0.01$) decrease only during the exacerbation phase but not during the remission or well-controlled period. Since AAG is smaller than albumin in molecular weight ($\text{AAG} \approx 44000$ vs $\text{albumin} \approx 69000$), one may assume that not only albumin but also AAG level may substantially decrease in patients with nephrotic syndrome due to an excessive urinary loss. Since the urinary excretion of AAG was not specifically assessed in the present study, no explanation for this observation can be offered.

We observed that approximately 26 and 14% of the total disopyramide binding at $3.0\text{ }\mu\text{g ml}^{-1}$ were attributed to the nonspecific binding(s) during the exacerbation and remission phases, respectively. In addition, the mean nonspecific binding parameter was significantly ($P < 0.05$) lower during the exacerbation phase than during the remission phase but the nonspecific binding parameter did not correlate with albumin. Based upon these findings, a possibility exists that unidentified component(s) other than AAG and albumin in the blood, although seemingly minor, might have somewhat contributed to the disopyramide binding and been affected by the change in the clinical status of our patients with nephrotic syndrome.

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