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Quantitative and qualitative binding characteristics of disopyramide in serum from patients with decreased renal and hepatic function

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1 Protein binding of disopyramide, binding capacities, affinity constants and serum concentrations of α_1-acid glycoprotein (AAG) were studied in five groups of patients. A: young healthy volunteers ($n = 8$); B: elderly patients with minor symptoms of ischaemic heart disease ($n = 9$); C: patients with cirrhosis of the liver and normal values of coagulation factors (II, VII and X), albumin and immunoglobulin G ($n = 8$); D: patients with cirrhosis and at least two abnormal of the previously mentioned values ($n = 9$) and E: eleven patients with severely impaired renal function.

2 Subfractions of AAG (Fr_1, Fr_2 and Fr_3) were determined by affinoimmunoelectrophoresis. AAG concentration was significantly ($P < 0.005$) elevated in group E patients and decreased ($P < 0.025$) in group D patients.

3 Fr_2 is probably associated with the high affinity, first binding site of disopyramide to AAG. Earlier observations of a reduced qualitative binding of disopyramide in patients with cirrhosis can be explained by a significant decrease in Fr_2 ($P < 0.001$) in group D patients.

4 The protein binding of disopyramide in patients with uraemia was significantly increased due to a significant ($P < 0.005$) increase in AAG concentration in spite of a smaller ($P < 0.025$) affinity constant.

5 Suggestions for therapeutic drug monitoring based on total serum concentrations are given.

Keywords disopyramide protein binding α_1-acid glycoprotein subfractions uraemia decreased hepatic function drug monitoring

Introduction

Disopyramide is a basic drug mainly bound to α_1-acid glycoprotein (AAG) in serum (Bredesen & Kierulf, 1984). The binding is concentration dependent within the therapeutic plasma concentration range (Cunningham *et al.*, 1977). α_1-acid glycoprotein is a heterogeneous protein composed by several subfractions each characterised by the structure of the sugar-part of the protein (Hansen *et al.*, 1984). Bonde *et al.* (1986) have recently demonstrated a qualitatively

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reduced binding of disopyramide to AAG (lower binding relative to the concentration of AAG at therapeutic serum concentration) in patients with hepatic insufficiency compared to an age-matched control group.

Haughey *et al.* (1985) have demonstrated a substantial decrease in the free fraction of disopyramide in patients on haemodialysis and renal transplant recipients. This could be attributed to a significant increase in the serum concentration of AAG.

The purpose of the present investigation was to study the influence of age, hepatic and renal insufficiency on the protein binding of disopyramide. To characterise the binding sites we have measured the total concentrations of AAG and quantitated each subfraction of the glycoprotein.

Methods

Serum from a total number of 45 persons was examined.

Group A ($n = 8$) Eight young healthy men with a mean age and weight of 31 years (range: 25–37 years) and 73.1 kg (range: 63–83 kg), respectively. None of the subjects received any medication or had an alcohol consumption of more than 25 g/day. One was a heavy smoker (> 20 cigarettes/day).

Group B ($n = 9$) Four elderly female and five male patients with minor symptoms of ischaemic heart disease. The patients received no medication apart from mild diuretics and nitroglycerine. The age and weight of the patients varied between 55–77 years (mean: 60 years) and 55–90 kg (mean: 69.0 kg), respectively. Four of the patients were smokers. None had an alcohol consumption of more than 25 g/day. The patients had normal biochemistry.

Group C ($n = 8$) Five male and three female patients with a histologically verified cirrhosis of the liver and normal values of coagulation factors (II, VII, and X), serum albumin, IgG and serum creatinine. The age and weight of the patients varied between 49 to 67 years (mean: 58 years) and 55 to 95 kg (mean: 74.6 kg), respectively. The patients were all smokers (6–20 cigarettes/day). One of the patients had a daily alcohol consumption of 100 g/day while the rest were not drinking at the time of the investigation. The patients received no medication apart from mild diuretics and vitamins.

Group D ($n = 9$) Three male and six female patients with a histologically verified cirrhosis of the liver and at least two abnormal values of the following: coagulation factors (II, VII and X< 0.7), serum albumin (< 450 μmol l^{-1}) and increased concentrations of IgG (> 14.5 g l^{-1}). The age and weight of the patients varied between 52 and 82 years (mean: 63 years) and 45–82 kg (mean: 59.9 kg), respectively. Serum creatinine was within normal range in all of the patients. Eight of the patients were smokers (5–25 cigarettes/day). One was a heavy drinker while the rest claimed to have an alcohol intake of less than 25 g/day. Medication comprised mild diuretics and vitamins.

Group E ($n = 11$) Four male and seven female patients with end-stage kidney disease, with an estimated mean glomerular filtration rate of 7.5 ml min^{-1} (range 1.5–10.0 ml min^{-1}), secondary to diabetic nephrosclerosis, chronic glomerulonephritis, amyloidosis, Schoenlein Henoch purpura, chronic interstitial or obstructive nephropathy. None of the patients was dialyzed. The majority of the patients had normal values of serum albumin and coagulation factors. The age of the patients varied between 19–69 years (mean: 50 years). The patients medication was numerous and included vitamins, prednisone, captopril, arteriolar dilatators, β-adrenoceptor blockers, diuretics, azathioprine and digoxin. None of the patients was a heavy drinker or smoker.

Protein binding

Protein binding was studied by equilibrium dialysis at a temperature of 37 °C (Bonde *et al.*, 1985). From each patient total serum concentrations of 0.4, 1.0, 2.0, 4.0, 10.0 and 20.0 μg ml^{-1} were prepared by adding disopyramide to the sample. The stock solution of disopyramide was pipetted into glass tubes and evaporated to dryness before serum was added. For each concentration the dialysis was made in duplicate. Analysis of disopyramide was made by liquid scintillation counting of [^{14}C]-disopyramide (supplied by Searle G. D., Skokie, Ill., U.S.A., specific activity 4.29 mCi $mmol^{-1}$), which was added to the buffer used (isotonic 0.05 M phosphate pH 7.40). The concentration of substance following the [^{14}C]-disopyramide is included in the above mentioned concentrations.

A modified Scatchard equation (Rosenthal, 1967) was fitted to the data from the protein binding experiments:

$$C_b = P_1 k_1 C_f/(1+k_1 C_f) + P_2 k_2 C_f/(1+k_2 C_f)$$

where C_b is the bound concentration, P_1 and P_2 are binding capacities (equivalent to serum con-

centration of binding protein presuming a single binding site ($n = 1$), k_1 and k_2 association constants for the two binding sites of disopyramide in serum and C_f the free concentration. The equation was fitted to the data by use of a nonlinear least square program using a RC-8000 computer.

As all binding curves levelled off and did not seem to approach zero at high drug concentrations the first binding site was assumed to represent AAG, while the second site had a very large capacity with a low affinity and might represent binding to other substances in serum. P_2 was therefore fixed to a value of 500 μmol l^{-1} in the subsequent curve fitting, and the results of the binding to the second site are given as per cent bound to this site at high serum concentrations when binding to the first site is of only minor importance.

The subfractions of AAG were analyzed by affinoimmunoelectrophoresis (Hansen *et al.*, 1984). This technique using the lectin can reveal differences in the glycan structures of an otherwise homogenous protein (Bøg-Hansen, 1983). During first dimension electrophoresis the lectin retards the molecules with glycans corresponding to the affinity of the lectin. Using the lectin concanavalin A we have thus found AAG to consist of three microheterogenous types, each characterised by the antennary structure of the glycan part of the glycoprotein (Hansen *et al.*, 1984). By this method we were able to quantitate the relative occurrence of the three subfractions: Fr_1, Fr_2 and Fr_3.

The data were subjected to statistical analysis by use of the paired *t*-test. A *P* value less than 0.05 was considered significant. The patients were all informed of the nature of the study which was approved by the local Ethics Committee.

Results

Table 1 shows the binding characteristics of disopyramide in the five patients group. The binding capacity was increased ($P < 0.025$) and the binding affinity decreased $P < 0.025$) in the patients with impaired renal function compared to group A, B and C. In contrast, cirrhotic patients with abnormal biochemical data had a decreased P_1 ($P < 0.01$) and an unchanged k_1 compared to group A, B and C. Binding capacity was increased ($P < 0.025$) in elderly patients compared to young healthy volunteers. Binding to the second binding site was significantly ($P < 0.001$) increased in the uraemic patients compared to the other groups. The table also shows the calculated values for total serum concentrations of disopyramide corresponding to the

Table 1 Capacity (P_1) and association constants (k_1) of the first binding site and the binding to the second site of binding of disopyramide in serum from each of the five groups. Values are given in mean ± s.d. Total concentrations (C_{tot}) calculated for a free concentration (C_f) of 3 and 6 μmol l^{-1} of disopyramide, respectively. C_b denotes the percentage of bound disopyramide at a free concentration of 6 μmol l^{-1}.

Patient group	*A: Young healthy volunteers*	*B: Patients with ischaemic heart disease*	*C: Cirrhotic patients with normal biochemical data*	*D: Cirrhotic patients with abnormal biochemical data*	*E: Patients with impaired renal function*
P_1 (μmol l^{-1})	5.86 ± 1.09[3]	8.18 ± 2.74[2,3,4]	6.53 ± 1.99	4.09 ± 1.50[4]	13.00 ± 4.81[2]
$k_1 \times 10^{-6}$ ($l\ mol^{-1}$)	1.43 ± 0.33	1.37 ± 0.19[2]	1.38 ± 0.27	1.53 ± 0.30	1.06 ± 0.37[2]
Binding to the second site (%)	22.2 ± 4.5	23.3 ± 5.7[1,4]	21.1 ± 11.5	14.4 ± 7.1[1]	36.8 ± 10.2[4]
$C_{tot.}$ ($C_f = 3$) (μmol l^{-1})	8.6	10.5	9.1	6.9	14.4
$C_{tot.}$ ($C_f = 6$) (μmol l^{-1})	13.0	15.1	13.4	10.7	20.7
C_b (%)	53.8	60.3	55.2	43.9	71.0

1: $P < 0.05$
2: $P < 0.025$
3: $P < 0.025$
4: $P < 0.001$

free concentration of disopyramide of 3 and 6 μmol l^{-1}, respectively.

In Table 2 the concentrations of AAG and the relative contribution of the three subfractions are given. Total serum concentration of AAG was significantly ($P < 0.005$) increased in the patients with impaired renal function compared with the other groups. Serum concentration of AAG was significantly ($P < 0.025$) lower in the patients with cirrhosis and abnormal biochemical data when compared to the age compatible group B. In the patients with impaired renal function the relative contribution of the three subfractions differed from group B as Fr_1 was significantly ($P < 0.025$) decreased while Fr_3 was increased ($P < 0.005$) and no difference in Fr_2 could be demonstrated. In group D patients, however, Fr_1 was increased ($P < 0.0005$) while Fr_2 and Fr_3 were significantly ($P < 0.001$) decreased.

Linear regression between the concentration of total AAG and the three subfractions against the capacity of the first binding site resulted in high coefficients of correlation ($r > 0.9$) in the four situations, thus not being able to point out one fraction(s) representing the first binding site.

Discussion

Our findings of an increased concentration of AAG (Table 1) in patients with impaired renal function are in agreement with the observations of Haughey *et al.* (1984) and Weeke *et al.* (1971) and might be attributed to a decreased rate of degrading of the glycoprotein in the kidneys or an increased formation rate. The low values of AAG in patients with cirrhosis and abnormal biochemical data are in accordance with the observations of Barre *et al.* (1984), Cleve & Strohmeyer (1967) and Hiramatsu *et al.* (1976), and can be explained by a reduced formation rate as AAG is exclusively produced in the liver.

It has previously been demonstrated that AAG is composed of three well defined subfractions (Hansen *et al.*, 1984). Our results of a decreased concentration of Fr_2 in patients with cirrhosis and abnormal biochemical data and an increase in Fr_3 in uraemic patients has not been described previously.

It is noticed from Tables 1 and 2 that the binding affinity to the second binding site is high in the group with high values of Fr_3 (group E) while the binding is low with low values of Fr_3 (group D). This might mean that Fr_3 (maybe in conjunction with other acute phase reactants) could make up the second binding site of disopyramide to AAG.

The percentage of bound disopyramide in serum from patients with impaired renal function was significantly increased compared with an age compatible control group (percentage bound at a free concentration of 6 μmol l^{-1} in group B and E, respectively: 60.3 & 71.0). This is in accordance with the results of Haughey *et al.* (1985) and Grossman *et al.* (1982) who demonstrated an increased binding of lignocaine, another basic antiarrhythmic drug, to AAG in patients with renal failure. The increased protein binding of disopyramide in patients with renal failure is caused by the high concentration of AAG—an acute phase reactant known to increase during a variety of stress conditions (Perucca *et al.*, 1985). In the study of Grossman *et al.* (1982) no dif-

Table 2 Concentration in serum of α_1-acid glycoprotein (AAG) and the relative contribution of each of the three subfractions of the protein (Fr_1; Fr_2 and Fr_3). Values given as mean ± s.d.

Patient group	*A: Young healthy volunteers*	*B: Patients with ischaemic heart disease*	*C: Cirrhotic patients with normal biochemical data*	*D: Cirrhotic patients with abnormal biochemical data*	*E: Patients with impaired renal function*
Serum concentration of AAG (μmol l^{-1})	18 ± 4	25 ± 11[1,2]	22 ± 6	15 ± 6[1]	52 ± 17[2]
Fr_1 (%)	42.8 ± 5.8	45.4 ± 5.2[1,4]	46.8 ± 8.2	59.1 ± 6.0[4]	38.1 ± 8.2[1]
Fr_2 (%)	41.8 ± 2.0	41.0 ± 1.9[3]	41.8 ± 3.8	35.6 ± 3.6[3]	39.4 ± 2.7
Fr_3 (%)	15.4 ± 4.4	13.7 ± 4.3[2,3]	11.5 ± 4.8	5.2 ± 3.3[3]	22.5 ± 6.1[2]

1: $P < 0.025$
2: $P < 0.005$
3: $P < 0.001$
4: $P < 0.0005$

ference in the binding affinity of lignocaine to AAG could be demonstrated. The correlation between the binding ratio and AAG, however, was obtained using data from a variety of kidney diseases (nephrotic syndrome, uraemia and renal transplants) making a comparison with our patients (exclusively uraemics) less meaningful.

The protein binding of disopyramide in renal failure is increased comparatively less than the increase in binding capacity (P_1). This might be caused by the significant lower binding affinity (k_1). The concept of an increased (P_1) quantitative but a reduced qualitative (k_1) binding has to our knowledge not been established for other basic compounds than disopyramide in renal failure.

In patients with cirrhosis and abnormal biochemical variables serum concentration of AAG is reduced (Table 1) resulting in low binding of disopyramide. In a previous study (Bonde *et al.*, 1986) we have demonstrated that the binding of disopyramide in such patients is significantly lower, even after correcting for differences in AAG concentrations, strongly suggestive of a qualitatively reduced binding of disopyramide in patients with cirrhosis and abnormal biochemical data. From the present study the reduced binding in group D patients can be explained by a reduced concentration of the alleged main binding fractions (Fr_2 and Fr_3).

The binding capacity was significantly greater ($P < 0.025$) in the elderly patients compared with the young healthy volunteers. This increase, however, is parallel to the increase in AAG concentration, implying an unaltered qualitative binding of disopyramide to AAG in the elderly. These results are in accordance with the results of Davis *et al.* (1985), who could not demonstrate an age related difference in the protein binding of lignocaine.

In Table 2 total serum concentrations of disopyramide at calculated free concentrations of 3 and 6 μmol l^{-1} are given. These free concentrations represent the lower and upper limits of the recommended therapeutic range based on total serum concentrations (Niarchos, 1976). The clinical implication of our findings is consequently that the therapeutic range based on *total serum* concentrations in patients with cirrhosis and abnormal biochemical data is suggested to be approximately 6.9–10.7 μmol l^{-1} and approximately 14.4–20.7 μmol l^{-1} in patients with severely impaired renal function compared an age compatible control group (10.5–15.1 μmol l^{-1}).

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