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Relationship between α_1-acid glycoprotein and plasma binding of disopyramide and mono-*N*-dealkyldisopyramide

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1 Highly purified serum albumin did not bind either disopyramide (DP) or mono-*N*-dealkyldisopyramide (MND). The unbound fraction of DP and MND in highly purified serum α_1-acid glycoprotein (AAG) at 0.5 g/l was 57 and 62 and at 2.0 g/l 19 and 30% respectively.

2 Unbound DP and MND were measured in spiked plasma (10 μmol/l of DP or MND), from 60 patients, having AAG concentrations varying from 0.4 to 3.0 g/l. Unbound drug varied from 13 to 58 and from 24 to 62% for DP and MND, respectively, and was inversely related to the plasma concentration of AAG ($r = -0.9016$, $r = -0.9157$).

3 A linear relationship was found between the binding ratio (moles bound divided by moles unbound) and the plasma concentration of AAG for both DP ($r = 0.9199$) and MND ($r = 0.9270$), whereas no relationship was found between the binding ratios of DP or MND and the plasma concentrations of total protein, albumin, haptoglobin, α_1-antitrypsin or the immunoglobulins IgG, IgA or IgM.

4 In patients on DP maintenance therapy, a linear relationship was found between percent unbound DP and the plasma concentration of DP in samples with similar AAG concentrations. Furthermore, a linear relationship was found between the binding ratio of DP and the plasma concentration of AAG in samples with similar DP concentrations.

5 The present findings support the concept that AAG is the major serum protein responsible for the binding of DP and MND.

Keywords α_1-acid glycoprotein disopyramide protein binding

Introduction

Disopyramide (DP) is an antiarrhythmic drug with an assumed therapeutic range of 6-15 μmol/l (Garfein, 1982; Follath *et al.*, 1983). About 55% of the administered dose of DP is eliminated unchanged, primarily by the kidneys. The main metabolic pathway of DP is dealkylation to the pharmacologically active metabolite, mono-*N*-dealkyldisopyramide, and about 25% of the administered drug is excreted by the kidneys as MND. The serum concentration of MND was previously thought to be only about 10% of that of the parent drug. However, recent studies have shown that the steady-state concentrations of MND in some patients on maintenance treatment with DP, were even higher than that of the parent drug (Aitio, 1981; Bredesen *et al.*, 1982).

The plasma protein binding of both DP and MND shows a wide intersubject and concentration dependent variability even within the assumed therapeutic range (Meffin *et al.*, 1979; David *et al.*, 1980; Lima *et al.*, 1981; Bredesen

et al., 1982). High concentrations of MND change the protein binding of DP and *vice versa* (Bredesen *et al.*, 1982). Thus measuring total drug concentration of DP or MND cannot be used to predict the individual free drug concentration of neither DP nor MND.

A previous study indicated that albumin was the main binding protein of DP (Chien *et al.*, 1974). Recent studies, however, in this laboratory and others (Lima *et al.*, 1981; David *et al.*, 1983; Pike *et al.*, 1983) have shown that DP binds mainly to the acute-phase protein, α_1-acid glycoprotein (AAG). The plasma concentration of AAG may be elevated in various disease states, associated with a variation in the binding of basic drugs (Fremstad *et al.*, 1976; Piafsky *et al.*, 1980; David *et al.*, 1983).

The purpose of the present study was to investigate the relationship between DP and MND binding and the plasma concentration of AAG.

Methods

The binding of DP and MND (DP and MND, Roussel Labs, Wembley Park, London, UK) to albumin (Albumin Kabi, Stockholm, Sweden) and to α_1-acid glycoprotein (AAG) (AAG Behringwerke, Marburg Lahn, Germany) was tested by spiking each preparation with DP or MND, using equilibrium dialysis and gas chromatographic determination as described previously (Bredesen, 1980; Bredesen *et al.*, 1982). The AAG concentration in the albumin solution tested was less than 0.05 g/l. The albumin concentration in the AAG solution tested was less than 0.01 g/l. The serum protein binding of DP or MND were studied in 60 *in vitro* spiked patient samples with different concentration of AAG. Thirty-one males aged 16–89 years (mean 78), and 29 females aged 28–88 years (mean 76). The binding of DP and MND was tested by spiking each sample with 10 μmol/l DP or MND. Concentrations of total protein, haptoglobin, α_1-antitrypsin and the immunoglobulins IgA, IgG and IgM were measured in each sample using nephelometric methods, and albumin by a dye (BCP) binding method (Pike & Skuterud, 1983).

α_1-acid glycoprotein was determined immunochemically by nephelometry. Serum was diluted 1/100 with saline (200 μl), antibody (DAKO-immunoglobulins, Denmark) (40 μl) added, the volume adjusted to 800 μl with saline, and the mixture left at room-temperature for 45 min, prior to reading in the Beckman Immunochemistry System as an end-point determination. Standards were prepared by appropriate dilutions of the LN-standard serum from Behringwerke (Marburg Lahn, Germany). A total of 30–40 samples were performed per hour. Concentrations down to 0.01 mg AAG/l were detected with a coefficient of variation of 5.5%. The serum binding of DP was also measured in 60 samples, 34 males, aged 31–82 years (mean 69) and 26 females, aged 42–86 years (mean 72), all on maintenance therapy with DP. All patients had used DP for more than 2 weeks, and the samples were drawn just before the dose of the drug.

Results

The binding of DP and MND to purified albumin and α_1-acid glycoprotein (AAG) are shown in Table 1. Neither DP nor MND bound to albumin whereas both DP and MND bound to AAG in varying degree depending on the protein concentration. The binding was in the same order of magnitude as for patient samples with corresponding AAG concentrations (Figure 1).

Table 1 Binding of disopyramide (DP) and mono-*N*-dealkyldisopyramide (MND) to albumin and α_1-acid glycoprotein (AAG).

Concentration	*% unbound DP*	*% unbound MND*
AAG 0.5 g/l	57	62
AAG 2.0 g/l	19	30
Albumin 40 g/l	100	102

The percent of unbound drug in the 60 *in vitro* spiked samples tested varied from 13 to 58 (mean 24) and 24 to 62 (mean 41) for DP and MND respectively. The AAG concentrations in the 60 *in vitro* spiked samples varied from 0.4 to 3.0 g/l. There was a negative linear relationship between the AAG concentration and the percent unbound DP ($r = -0.9016$) and MND ($r = -0.9157$) (Figure 1 and 2, closed circles). The open circles in Figure 1 and 2 show the unbound fraction of DP and MND in one sample diluted with different volumes of the highly purified albumin, 50 g/l. The AAG concentration in the undiluted sample was 2.5 g/l. In the diluted samples the concentrations ranged from 2.4 to 0.1 g/l. These data indicate a nonlinear relationship between the binding of DP and MND and the AAG concentration, a trend which is difficult to discover in the data from the 60 *in vitro* spiked samples.

In Figures 3 and 4 the data therefore are presented as the relationship between the binding ratio (moles bound divided by moles

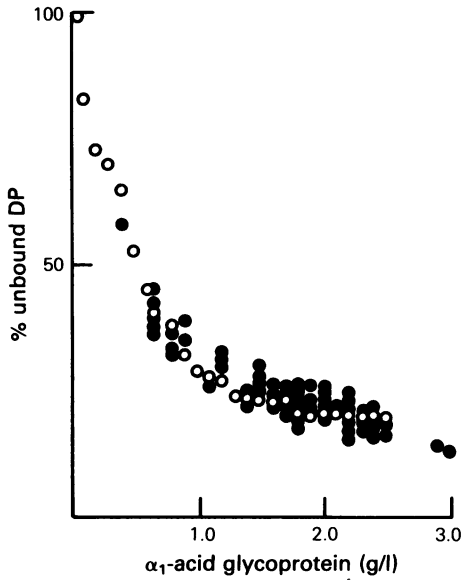

Figure 1 Relationship between α_1-acid glycoprotein (AAG) concentration and percent unbound disopyramide (DP). Closed circles patients samples (r = −0.9016). Open circles one patient sample (AAG concentration 2.5 g/l) diluted with purified human albumin (concentration 50 g/l).

unbound) and the AAG concentration. This relationship should be linear if the dissociation constant for the drug protein complex is much higher than the molar concentration of free drug (Nilsen *et al.*, 1978). A linear relationship was found between the AAG concentration and the binding ratio. For DP and MND respectively the relationship was 0.9199 and 0.9270 for the 60 *in vitro* spiked samples, (closed circles in Figure 3 and 4), 0.9792 and 0.9904 for the diluted samples (open circles in Figure 3 and 4) (0.9514 and 0.9485 for all points).

Poor or no relationship were found between the binding ratio of DP and MND and the serum concentration of total protein (r_{DP} = 0.25, r_{MND} = 0.18), albumin (r_{DP} = 0.29, r_{MND} = 0.15), haptoglobin (r_{DP} = 0.14, r_{MND} = 0.18), α_1-antitrypsin (r_{DP} = 0.37, r_{MND} = 0.28) or the immunoglobulins IgG (r_{DP} = 0.27, r_{MND} = 0.31), IgA (r_{DP} = 0.01), r_{MND} = 0.11) and IgM (r_{DP} = 0.34, r_{MND} = 0.36). In the 60 samples tested no relationship was found between binding and age (r_{DP} = 0.08, r_{MND} = 0.11). To investigate the relative importance of the different proteins and age on DP and MND binding a multiple regression analysis was performed of the binding ratio against the different proteins and age. All the variables explained 90% and 91% respectively of the

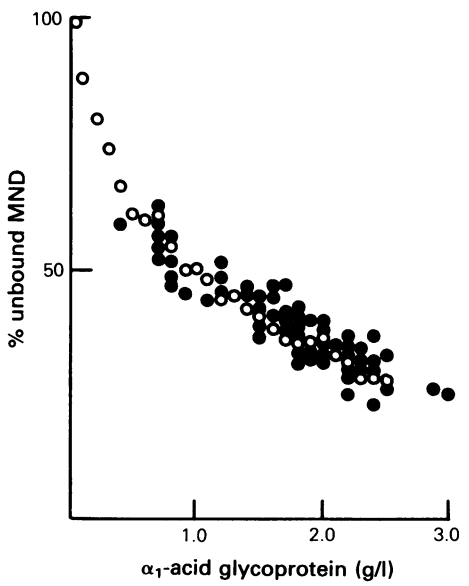

Figure 2 Relationship between α_1-acid glycoprotein (AAG) concentration and percent unbound mono-*N*-dealkyldisopyramide (MND). Closed circles patient samples (r = −0.9157). Open circles one patient sample (AAG concentration 2.5 g/l) diluted with purified human albumin (concentration 50 g/l).

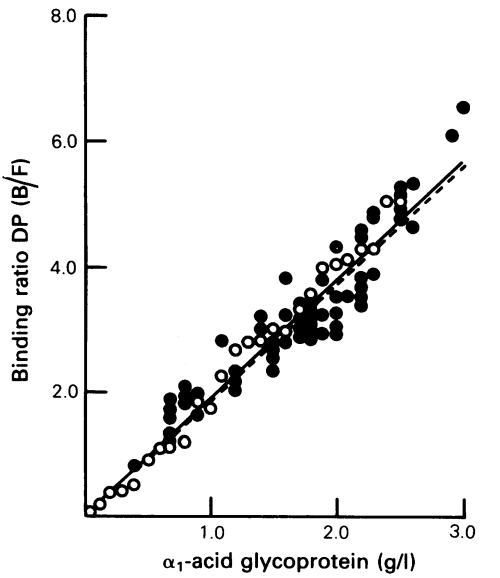

Figure 3 Relationship between α_1-acid glycoprotein (AAG) concentration and the binding ratio of disopyramide (DP). Open circles one patient sample (AAG concentration 2.5 g/l) diluted with human albumin. Closed circles patient samples (r = −0.9199, broken line: y = 1.9 × +0.02). All points (r = 0.9488, solid line: y = 1.9 × +0.03).

total binding variation of DP and MND, and of these explained variations, 94% was due to AAG alone for both DP and MND.

Large variations in the protein binding of DP was found in samples from the 60 patients on maintenance therapy with DP. The total concentration of DP ranged from 2.1 to 12.0 μmol/l, mean 6.6 μmol/l, and the percent unbound fraction from 5 to 37, mean 17 (Figure 5). Poor relationship between DP concentration and percent unbound drug was found (r = 0.1018, all points, Figure 5). However, the concentration of AAG in the 60 samples ranged from 0.5 to 2.4 μmol/l, mean 1.3 μmol/l, and a linear relationship was found between percent unbound DP and the total DP concentration in samples with similar AAG concentrations. In samples where the AAG concentrations range from 0.5 to 0.9 g/l (Figure 5, open triangles), from 1.0 to 1.9 g/l (Figure 5, closed circles), and from 2.0 to 2.4 g/l (Figure 5, open circles), the relationship were 0.8361, 0.7344 and 0.7396 respectively. No correction was made for the possible effect on MND on the DP binding. The MND concentrations ranged from 0.4 to 11.3 μmol/l, mean 3.1 μmol/l. A linear relationship was also found between the binding ratio of DP and the plasma concentration of AAG in the samples where DP concentrations ranged from 2.1 to 4.9 μmol/l (r = 0.8122, Figure 6, open circles), from 5.1 to 7.9 μmol/l (r = 0.8378, Figure 6, closed circles), from 8.1 to 10.8 μmol/l (r = 0.7818, Figure 6, open triangles) and from 11.4 to 12.0 (r = 0.9911 Figure 6, closed triangles). All points, r = 0.4264.

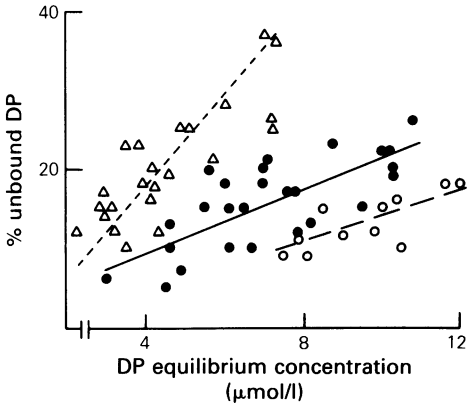

Figure 5 Relationship between percent unbound fraction of disopyramide (DP) and disopyramide equilibrium concentration in 60 patients on maintenance therapy with disopyramide. Open triangles α_1-acid glycoprotein (AAG) concentration 0.5 to 0.9 g/l (r = 0.8361, y = 3.7 × +3.4). Closed circles AAG concentration 1.0 to 1.9 g/l (r = 0.7344, y = 1.9 × + 2.1). Open circles AAG concentration 2.0 to 2.4 g/l (r = 0.7396, y = 1.6 × −2.4).

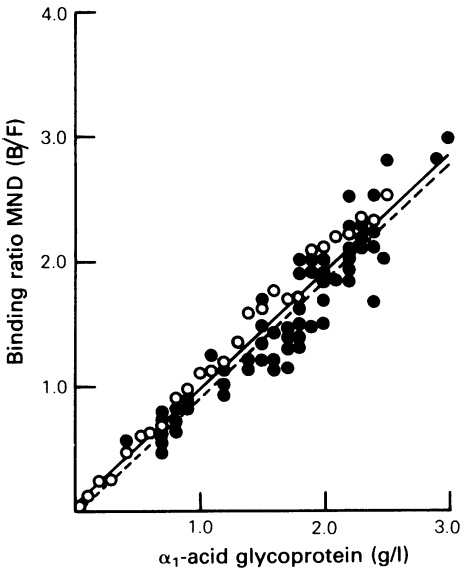

Figure 4 Relationship between α_1-acid glycoprotein (AAG) concentration and the binding ratio of mono-*N*-dealkyldisopyramide (MND). Open circles one patient sample (AAG concentration 2.5 g/l) diluted with human albumin. Closed circles patient samples (r = 0.9270, broken line: y = 0.9 × −0.01). All points (r = 0.9485, solid line: y = 0.9 × +0.02).

To investigate the relationship between DP binding, AAG, total DP and total MND concentrations, a multiple regression analysis was performed of percent unbound DP against AAG, total DP and total MND concentrations. The three variables together explained 64% of the DP binding variations, while each alone contributed to only 17%, 2%, and 1% respectively. The regression equation describing the relationship between percent unbound DP and AAG, total DP and total MND concentrations is as follows: Percent unbound DP = 2.2·DPcons. + 0.8·MNDcons. − 11.7·AAGcons. + 17.8 (r = 0.8021).

Discussion

Wide interindividual binding variations of DP have been reported by a number of authors (Aitio, 1981; Bredesen *et al.*, 1982; David *et al.*, 1983). The use of total plasma concentrations of DP to dose adjust problem patients may fail if binding variations are not taken into account.

To explain the binding variations of DP and MND the relationship between DP and MND

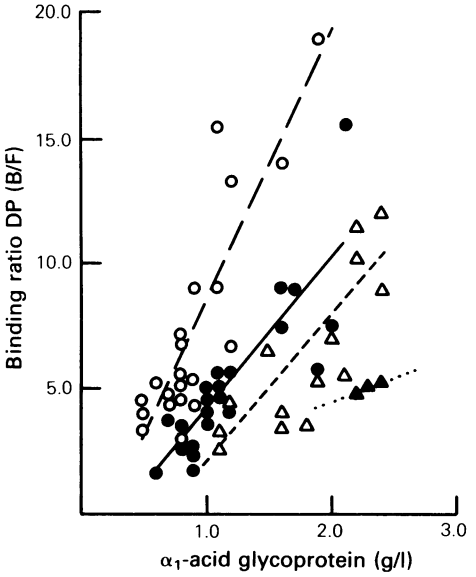

Figure 6 Relationship between α_1-acid glycoprotein and the binding ratio of disopyramide (DP). Open circles, DP concentration 2.1 to 4.9 μmol/l (r = 0.8122, y = 10.9 × −2.5). Closed circles, DP concentration 5.1 to 7.9 μmol/l (r = 0.8378, y = 6.0 × −1.9). Open triangles, DP concentration 8.1 to 10.8 μmol/l (r = 0.7818, y = 6.1 × −4.2). Closed triangles, DP concentration 11.4 to 12.0 μmol/l (r = 0.9966, y = 1.8 × +1.0).

binding and the concentration of the acute-phase protein α_1-acid glycoprotein (AAG) have been investigated.

Conflicting results have been published on the binding proteins of DP. Thus, Chien *et al.* (1974) reported that albumin was the main binding protein whereas Lima & Salzer (1981) reported that only 5–10% was bound to albumin. A recent study, however, showed that separate removal of albumin from serum did not affect the binding of DP. Separate removal of AAG, on the other hand, abolished the binding, thus indicating that AAG alone may be responsible for the binding of DP (Pike *et al.*, 1983). The lack of binding of DP and MND to purified albumin and the binding to purified AAG together with the close relationship between the AAG concentration and the binding of both drugs, strongly indicates that this protein is the serum protein binding DP and MND. This is also substantiated by extrapolation of the regression line (Figure 3, 4 and 6) of the relationship between the binding ratio of DP and MND and the AAG concentration to the abscissa, which indicates that neither DP nor MND would be bound in samples where AAG is absent. The finding of AAG as the main binding protein of DP as well as the lack of relationship between drug binding and other proteins tested, is in accordance with the results published by Holt *et al.* (1983), Johnston *et al.* (1983) and David *et al.* (1983).

Similar binding characteristics as for DP were found for MND. In a previous study (Bredesen *et al.*, 1982) it was also found that DP and MND were competing for similar binding sites on the proteins.

It is known that a wide variation in the AAG concentration may occur in a variety of diseases, associated with variation in the binding of basic drugs (Fremstad *et al.*, 1976; Piafksy *et al.*, 1980; David *et al.*, 1983). Thus, the variation in the binding of both DP and MND may be considerable even within the same individual. Therefore it may be almost impossible to predict the pharmacological active free drug concentration measuring only the total concentration. In the present study the binding data found in patients on maintenance therapy with DP substantiate this (Figure 5). As seen, a wide variety in the drug binding was found, with up to six-fold variance in free drug concentration at the same total concentration. No concentration dependent binding was found in these samples, evidently because of the wide variation in the AAG concentration in the different samples. However, as seen from Figure 5, concentration dependent protein binding was found in samples from patients on DP therapy, as for healthy subjects (Bredesen *et al.*, 1982) when the AAG concentrations in the samples were similar. Another explanation of the wide variation in the drug binding data shown in Figure 5, may be the possible effect of high concentrations of MND on the binding of DP (Bredesen *et al.*, 1982). Fourteen of the 60 samples had higher concentrations of MND than DP.

As for *in vitro* spiked samples, a linear relationship was also found between DP binding and the AAG concentration in samples from patients on maintenance therapy with DP when DP concentrations were similar (Figure 6). The relationship between binding and the AAG concentration is somewhat better for the spiked samples, probably due to large variations in MND concentrations in the samples from patients on maintenance therapy. Multiple regression analysis showed that both AAG, total DP and total MND concentrations have to be considered to predict the unbound fraction of DP.

The results of this study indicate that AAG is the major protein, and probably the only one, responsible for the binding of DP and MND. In addition to the variation in AAG concentration, the wide variety of the DP binding is also

determined by the concentration of both DP and MND in plasma. An attempt to establish plasma concentration/effect relationship based on free drug concentration should be carried out. Measuring free drug concentration is time consuming and not suitable for clinical monitoring. However, these results indicate that measurement of the AAG concentration together with both DP and MND concentration should be a useful guide to predict the unbound pharmacologically active concentration of DP.

The multiple regression analysis was done by Svein Børre Mogensen, Institute for Medical Statistics, Ulleval Hospital.

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