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BINDING OF QUINIDINE IN SERA WITH DIFFERENT LEVELS OF TRIGLYCERIDES, CHOLESTEROL, AND OROSOMUCOID PROTEIN

ODD G. NILSEN, PAUL LEREN, INGVAR AAKESSON and STEN JACOBSEN

Institute of Pharmacology, University of Oslo, and Ullevaal Hospital, Medical Out Patient Clinic and Medical Department VII, Blindern, Oslo 3, Norway

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Abstract—Serum binding of quinidine was determined *in vitro* by equilibrium dialysis in sera from twenty-five healthy individuals. The sera had different levels of triglycerides, cholesterol and orosomucoid (α_1-acid glycoprotein), but with small variations in serum albumin concentration. Binding ratio (bound/free) and per cent binding varied from 2.0 to 5.4 and from 67.1 to 84.3% respectively. Binding ratios were linearly related to serum concentration of triglycerides ($r = 0.437$, $P < 0.05$), cholesterol ($r = 0.400$ $P < 0.05$) and orosomucoid ($r = 0.841$, $P < 0.001$), but not to serum concentrations of albumin. When five persons with orosomucoid serum levels exceeding the accepted normal range were excluded, a better linear relation was observed for the triglycerides ($r = 0.765$, $P < 0.001$). The observed binding ratios were linearly related to binding ratios calculated from previous observations on binding parameters of lipoprotein and albumin fractions of human sera when all persons were included ($r = 0.465$, $P < 0.05$) and when those with high levels of orosomucoid were excluded ($r = 0.753$, $P < 0.001$).

Albumin has been rated as the main quinidine binding protein in human serum [1–4]. However, 30–35 per cent of the quinidine bound in serum is confined to lipoproteins compared with 65–70 per cent bound to serum albumin. Additionally, the binding of quinidine by the acute phase protein orosomucoid in post operative patients was recently proposed [14]. Some observations indicated that variations in binding of quinidine were not related to serum albumin levels [5].

The majority of patients treated with quinidine have probably serum levels of albumin within the normal range, while serum concentrations of triglycerides and cholesterol reflecting the levels of lipoproteins can be expected to vary more.

The purpose of this work has been to investigate the variation in quinidine binding in sera from normal individuals and the possible relation to serum concentrations of cholesterol, triglycerides, orosomucoid and albumin.

MATERIALS AND METHODS

Serum. Blood was obtained from twenty five healthy volunteers (29–54 yr old) with different levels of serum triglycerides and cholesterol. These volunteers had not taken any drugs the last month before blood samples were obtained. Venous blood was obtained after fasting overnight. The blood sample was left for 1 hr at room temperature and then centrifuged at 1100 *g* for 30 min at room temperature. Determinations of cholesterol and triglycerides were carried out the same day. The serum was stored at −20° until binding experiments were performed.

Chemicals. Quinidine hydrochloride was supplied by the Norwegian Drug Monopoly, Oslo, and ^{3}H-labelled quinidine with a specific activity of 500 mCi/m-mole was supplied by Buchler and Co., Braunschweig, West Germany. The unlabelled compound contained 96.7% quinidine, the rest being dihydroquinidine. The tritiated quinidine had identical R_f values with the main spot of unlabelled quinidine when chromatographed in three separate solvent systems; aceton–methanol–chloroform, 2:2:6 (v/v), cyclohexan–chloroform–dietylamin, 5:4:1 (v/v), and methanol–conc. ammonia, 100:1.5 (v/v).

Binding experiments. Serum binding of quinidine was determined by equilibrium dialysis, using a dialysis membrane 20/32 (Union Carbide Corpn, Chicago, IL) clamped between two Perspex^R cells. 500 μl aliquots of serum and buffer were introduced into the cells. Labelled and unlabelled quinidine (1×10^{-8} moles), were added to the aliquot of serum, producing serum concentration close to the therapeutic level. Krebs–Ringer bicarbonate buffer [7], pH 7.35, was used and the dialysis was performed in an atmosphere of 5% (v/v) carbon dioxide. Cells were shaken for 18 hr at 20°. Serum and buffer were gassed with 5% (v/v) carbon dioxide prior to the equilibrium dialysis in order to achieve pH 7.35. All sera were diluted 15–18 per cent by equilibrium dialysis.

Concentration of quinidine. The radioactivity of the [^{3}H]quinidine isotope in serum and buffer was determined in a Packard Tri-Carb liquid scintillation spectrometer model 3330 operated at 5°. Fifty μl serum or buffer was added 10 ml of scintillation fluid [6]. The counting efficiency was 28.5 per cent in both buffer and serum samples.

Binding calculations. The protein binding in different sera could not be determined at the same concentration of free quinidine, because of the relative small volume of buffer used. Available methods which use larger buffer volumes were not used because of the too high consumption of the quinidine isotope and serum. The extent of binding was consequently expressed as per cent binding and as the ratio

between molar concentrations of bound quinidine, B, and free quinidine, F, in serum. This ratio is an expression for the extent of drug–protein interaction, and is linearly related to the molar concentration of protein, P, in serum, when the dissociation constant, K_D, for the drug–protein complex is much higher than molar concentration of free drug, as can be observed from the equation [8] based on the law of mass action:

$$B/F = \frac{n \times P}{K_D + F}$$

n is number of binding sites on the protein molecule being available for the drug. The linearity between B/F and P has been tested at several concentrations of F, and has proved to be valid for all.

The binding ratio of quinidine in serum containing several quinidine binding proteins, can be calculated from the modified equation:

$$B/F = \sum_{P=1}^{P=m} \frac{n_P \times P_P}{K_{DP} + F}$$

where P, having values from 1 to m, index all the quinidine binding proteins in serum. However, if a protein possesses more than one binding class for quinidine, m is a number exceeding the number of quinidine binding proteins.

All regression lines were computed as least-square regression lines with equal weight on each point.

Cholesterol and triglycerides. Isopropanol extracts of serum were analyzed in an Auto Analyzer II, according to the instructions given by the Technicon Instrument Corpn (work sheet AA II-23 and 24, May 1971) with the exception that the final amount of water was the same for standards and for the serum samples. Two separate determinations were performed on each sample. The coefficient of variation for the determinations of cholesterol and triglycerides were ±3.5 and ±6 per cent, respectively [13].

Albumin and orosomucoid (α_1-acid glycoprotein). The concentrations of serum albumin and serum orosomucoid were determined by an immunological technique [9] in a barbital and veronal buffer respectively, at pH 8.6, using 1% agarose (w/v). The gels contained 2% (w/v) antialbumin serum and 2.5% (w/v) antiorosomucoid serum, respectively (Copenhagen, Denmark). Serum standards were obtained from Behringerwerke, Germany. Ten μl samples were applied on a gel with a thickness of 2 mm.

Electrophoresis. Paper electrophoresis of serum was performed at room temperature in a barbital buffer containing 1% (w/v) albumin as described by Lees and Hatch [10]. The electrophoresis strips were dried at 110° for 20 min and then stained with Sudan schwartz B. This method produced narrow and well defined bands of serum lipoproteins, and the chylomicrons would appear as a discrete band at the point of application of serum [10]. Chylomicrons were not detected in the serum samples from the fasting individuals participating.

Serum concentrations of very low, low, and high density lipoproteins. The concentrations of the major lipid components of serum lipoproteins were evaluated from the serum concentration of cholesterol and triglycerides [11]. The relative amount of triglycerides in VLDL was taken as 54.2% (w/w), of cholesterol in LDL as 37.3% (w/w), and of cholesterol HDL as 12.1% (w/w) [12]. The average mol. wt of very low, low, and high density lipoproteins, designated VLDL, LDL, and HDL, were assumed to be 5.2×10^6, 2.3×10^6, and 2.5×10^5 respectively [12, 2].

RESULTS

The individual serum concentrations of albumin, cholesterol, triglycerides and orosomucoid, together with the serum binding of quinidine are given in Table 1. Great variation in levels of cholesterol and triglycerides can be observed, while the concentration of albumin varied less. However, serum concentration of orosomucoid varied 4-fold and the level was distinctly higher than the normal range (0.009–0.031 mmole/l°* in five individuals. The serum binding as judged from the binding ratio (B/F) and per cent binding varied from 2.0 to 5.4 and from 67.1 to 84.3 per cent respectively. The observed data for binding of quinidine in these sera, expressed as the binding ratio (B/F), were plotted against the corresponding concentration of triglycerides, cholesterol, orosomucoid, and albumin in Figs 1–4. The linear correlation between binding ratios and the respective serum concentrations of triglycerides, cholesterol, orosomucoid and albumin was evaluated. Linear correlation with binding ratios could be observed for all these except serum albumin as can be seen in Table 2. The correlation for cholesterol and triglycerides was higher when the individuals with high levels of orosomucoid were excluded. A multiple correlation [16] between binding ratio, orosomucoid and triglycerides for all individuals demonstrated a coefficient of 0.911, the correlation coefficient between orosomucoid and triglycerides was calculated to be 0.138 and between orosomucoid and cholesterol 0.186.

Concentration of very low, low, and high density lipoproteins and of albumin in the individual sera were estimated as described in Methods. These concentrations were applied to calculate quinidine binding in the sera, using the experimental binding parameters from normal human plasma [12]. These calculated binding ratios were then plotted against observed binding of quinidine in the individual sera in Fig. 5. A linear correlation between calculated and experimental values of quinidine binding ($y = -9.0 + 4.3\ x$, $r = 0.465$, $P < 0.05$) was observed. However, if those individuals with high serum concentration of orosomucoid were omitted, the correlation was better ($y = 0.2 + 1.0\ x$, $r = 0.753$, $P < 0.001$). The theoretical line $y = x$ is plotted on the Fig.

DISCUSSION

Quinidine is known to be bound to several serum proteins, as albumin, lipoproteins and orosomucoid [2, 6, 14]. The lipoprotein concentrations in sera from healthy individuals are known to vary much, but albumin and orosomucoid concentrations are expected to be within a normal range. The results

* Values given by Behringwerke for adults in central Europe.

Table 1. Serum concentrations of lipids, orosomucoid and albumin and binding of quinidine

	Serum* conc. (m-mole/l)				Binding ratio† of quinidine (B/F)	Per cent† binding $\left(\frac{B \times 100}{B+F}\right)$
	Triglycerides	Cholesterol	Albumin	Orosomucoid		
O.W.	2.53	7.38	0.52	0.032	3.09	75.5
H.B.	1.79	6.27	0.54	0.021	3.31	76.8
W.N.N.	4.26	8.96	0.49	0.012	2.97	74.8
R.C.K.	2.36	7.54	0.63	0.021	3.86	79.4
W.O.H.	3.20	9.38	0.57	0.020	3.00	75.0
L.G.	3.00	8.18	0.51	0.027	3.35	77.0
K.J.G.	2.43	7.46	0.50	0.050	4.91	83.1
Th.F.	1.66	5.78	0.55	0.020	2.89	74.3
E.E.	4.76	10.13	0.54	0.020	3.68	78.6
A.T.	2.39	6.32	0.59	0.036	3.61	78.3
K.S.	5.50	6.48	0.55	0.029	4.20	80.8
O.T.S.	4.03	8.83	0.59	0.026	3.71	78.8
F.L.	2.31	6.63	0.51	0.024	3.45	77.5
R.S.	1.91	9.48	0.54	0.014	2.55	71.8
K.M.	1.46	7.95	0.50	0.035	3.58	78.2
R.A.B.	2.54	7.02	0.44	0.017	3.00	75.0
H.S.	1.13	6.24	0.48	0.018	3.24	76.4
B.L.	3.24	6.92	0.51	0.029	3.98	79.9
C.G.	1.67	8.52	0.54	0.051	5.38	84.3
K.R.	3.05	6.58	0.53	0.042	4.93	83.1
O.F.	2.93	5.57	0.62	0.022	3.34	76.9
O.B.	0.55	2.85	0.56	0.018	2.31	69.7
D.F.	0.85	5.00	0.58	0.012	2.30	69.6
O.N.	0.78	5.13	0.49	0.013	2.20	68.9
L.S.	0.62	3.96	0.54	0.018	2.00	67.1
Range	0.55–5.5	2.85–10.13	0.44–0.62	0.012–0.051	2.0–5.38	67.1–84.3

* Mean values of two and three separate determinations of concentrations after equilibrium dialysis.

† B and F represent concentrations of bound and free quinidine respectively, after equilibrium dialysis.

in Table 1 demonstrate the observed interindividual variation in serum concentrations of the lipids, orosomucoid and albumin, and in the binding of quinidine in a small group of healthy individuals. The variations in serum concentrations of triglycerides and cholesterol are considerable but in a range that might be expected in healthy individuals and potential patients with cardiac diseases. The albumin concentration varied, as expected, little in healthy individuals. The levels of orosomucoid were, however, distinctly higher than normal range in five sera. Such increase in serum orosomucoid is usually seen in cases with inflammatory and neoplastic diseases, and in stress situations. These five individuals had no sign of such diseases.

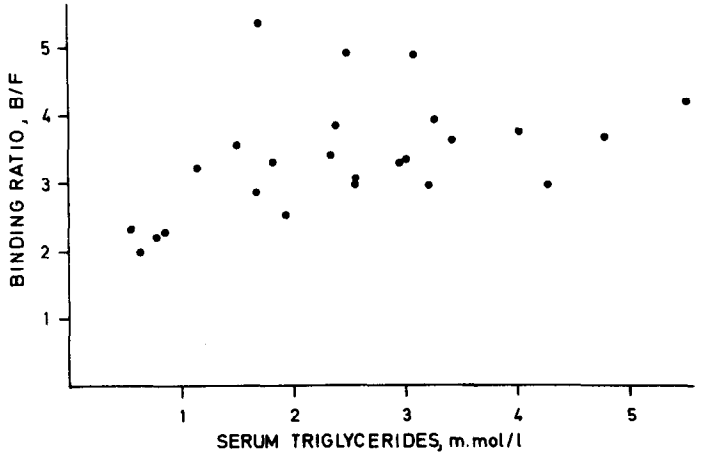

Fig. 1. Relation of concentrations of serum triglycerides to binding ratios (bound/free) of quinidine determined by equilibrium dialysis in twenty five individual sera.

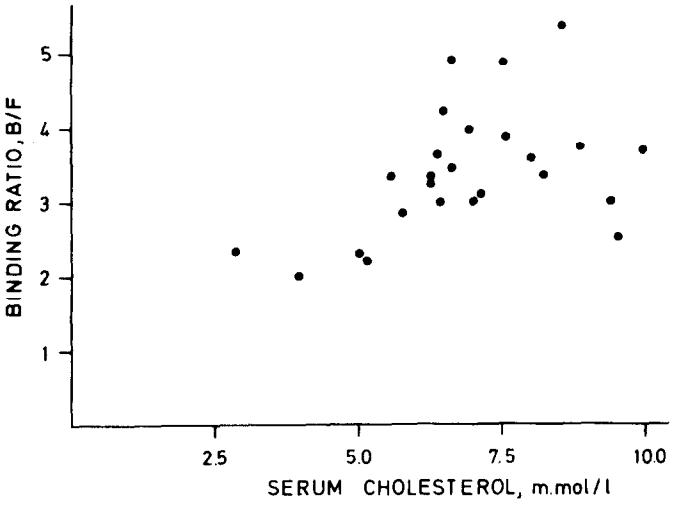

Fig. 2. Relation of concentrations of serum cholesterol to the binding ratios of quinidine.

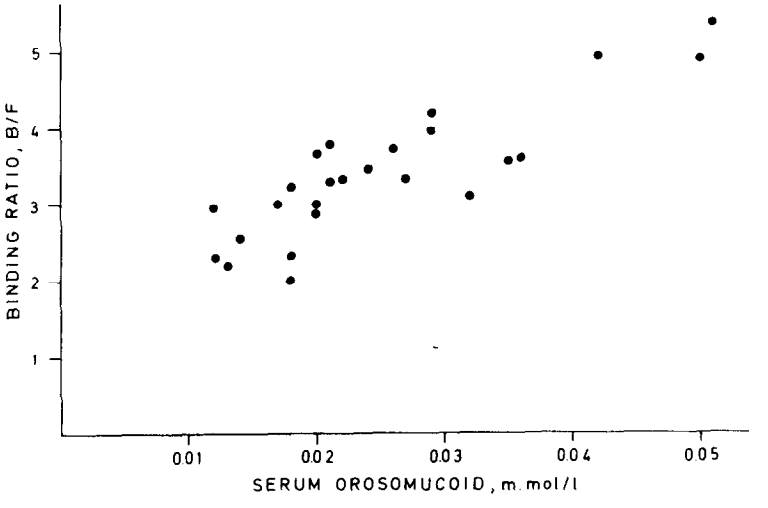

Fig. 3. Relation of concentrations of serum orosomucoid to the binding ratios of quinidine.

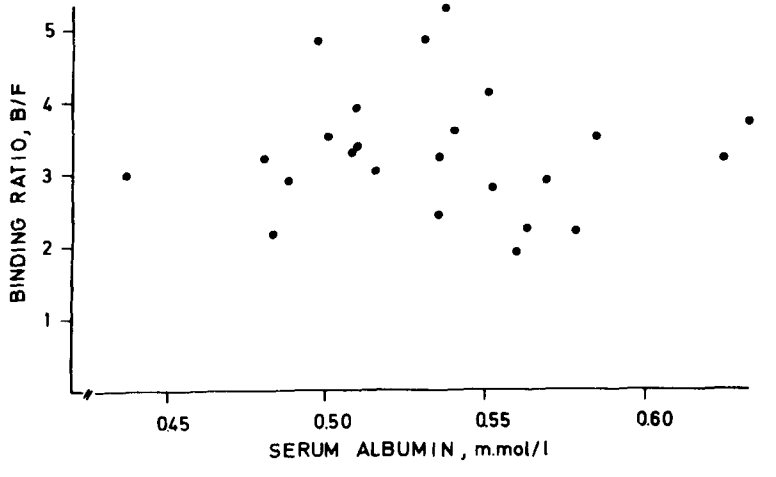

Fig. 4. Relation of concentrations of serum albumin to the binding ratios of quinidine.

Table 2. Correlation coefficients and P-values

	Triglycerides		Cholesterol		Orosomucoid		Albumin	
	r-value	P-value*	*r*-value	P-value	*r*-value	P-value	*r*-value	P-value
All individuals	0.437	<0.05	0.400	<0.05	0.841	<0.001	0.031	>0.1
All individuals except K.J.G., A.T., K.M., C.G. and K.R.	0.765	<0.001	0.474	<0.05	0.639	<0.01	0.156	>0.1

* Significance for r towards zero.

When the observed serum binding ratios for quinidine were plotted against the respective concentrations of triglycerides and cholesterol (Figs 1 and 2) linear correlations of moderate significance ($P < 0.05$) was demonstrated. Serum triglycerides and cholesterol are mainly confined to very low and low density lipoproteins respectively, but these lipids also are present in high density lipoproteins. Additionally, the relative distribution of these lipids to different lipoproteins is not constant [11]. These variables and the fact that the three lipoprotein fractions have different characteristics as quinidine binding molecules, may explain some of the rather low correlation coefficients.

The rather low correlation coefficient for lipid concentration and binding ratio is mainly caused by the variation in orosomucoid concentration. This protein seems to bind quinidine extensively [14] and the binding ratios for quinidine in the present sera is significantly ($P < 0.001$) correlated to the concentration of this protein (Fig. 3). These results demonstrate that when lipoproteins and orosomucoid are varying in the present range, orosomucoid has the dominant effect on the binding ratios. However, when those individuals with serum levels of orosomucoid exceeding the normal range were excluded, a better linear correlation (Table 2) was obtained for the triglycerides while only a smaller increase in correlation coefficient was observed for cholesterol. This demonstrates that in sera with an orosomucoid level within the normal range, quinidine binding is well related to serum levels of triglycerides ($P < 0.001$) but not to cholesterol.

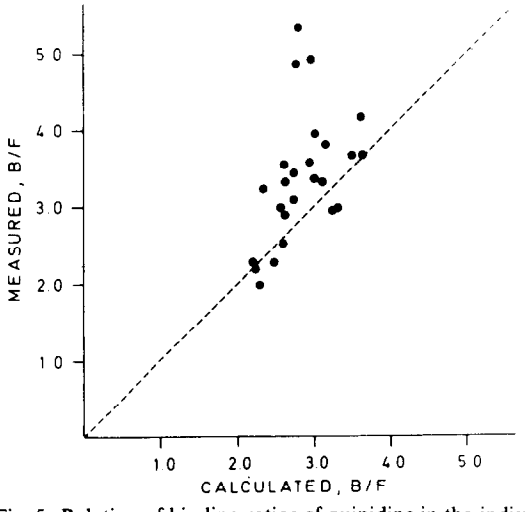

Fig. 5. Relation of binding ratios of quinidine in the individual sera to calculated binding ratios from binding characteristics of human serum protein fractions. The line represents the expected $x = y$.

The multiple correlation where variability in both triglycerides and orosomucoid were taken into account for all individuals, demonstrated that although 71 per cent of the variation in binding ratio was explained by referring to orosomucoid and 19 per cent was explained by referring to triglycerides, 83 per cent was explained by referring to both orosomucoid and triglycerides. This indicates that the serum level of very low density lipoproteins (VLDL) and orosomucoid is decisive for binding variability in human sera with albumin level within the normal range.

While there are linear correlation between binding ratios and triglycerides, cholesterol and orosomucoid, no significant correlation was observed between quinidine binding ratios and serum albumin concentration. This absence of significant correlation may be explained by the small variation in serum concentration of albumin.

The binding ratios for quinidine calculated from estimated levels of lipoproteins and albumin, and their binding characteristics of protein fractions obtained from normolipemic human sera [2], were compared with the present binding ratios (Fig. 5). The data demonstrate a deviation from the expected line ($y = x$) being more pronounced for sera with higher binding ratios. This observation indicates that these binding characteristics of normal serum protein fractions alone are not applicable on sera when concentration of other quinidine binding proteins as orosomucoid are higher than normal.

Concentration of orosomucoid was markedly increased in those sera exhibiting the highest quinidine binding ratio (Table 1). This protein has recently been proposed to bind quinidine extensively [14] and this may explain the weak correlation and the deviation from the ideal line. The linear correlation ($y = 0.2 + 1.0\,x$, $P < 0.001$) was observed when those sera containing high levels of orosomucoid was omitted, indicating a more general validity of the evaluated binding parameter for protein fractions. The intersection with the ordinate is probably due to the binding of quinidine to orosomucoid, and the correlation coefficient $r = 0.753$, is probably influenced by the variability of orosomucoid concentration within the normal range.

The rather good agreement in Fig. 5 without accounting for the binding to orosomucoid is probably caused by impurities of orosomucoid in the albumin fraction [2, 4]. Consequently, the binding parameters for albumin include binding to orosomucoid, but quantitatively to a smaller extent than in whole serum.

The observed variations in serum binding of quinidine producing a free fraction of quinidine ranging from 15.7 to 32.9 per cent are explained mainly by variation in the serum content of triglycerides and orosomucoid. As both serum lipids and orosomucoid protein are subject to great variation, serum binding of quinidine suggests conceivable pharmacodynamic and kinetic variations in patients receiving quinidine [15].

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