The results for the topical dose (Tables II and IV) show that ethoxzolamide could be detected in the untreated eye in 3 of 10 rabbits and in the treated eye in all of the rabbits. An outlier value of 2.08 ng/mg was determined for rabbit 1 in the untreated eye (Table III). The measurement in the untreated eye likely represents systemic absorption. The pooled treated eyes yielded iris/ciliary body levels of 2.13 ± 1.5 ng/mg (n = 10). The mean level is statistically lower ($p < 0.05$) than the mean value obtained for dosing at 6 mg/kg iv but not statistically different from tissue levels obtained at 2 mg/kg iv.

For an average-sized rabbit (2.5 kg), the minimum intravenous dose to produce a response (2 mg/kg) represents a 3.3-fold higher dose than that administered by the topical route. Thus, the nearly equal tissue levels shown in Tables III and IV for the two routes of administration suggest that the tissue levels detected from the smaller topical dose are, at least in part, a consequence of corneal transport and not primarily systemic absorption. Although our results do not differentiate between the direct and systemic effects of the drug, they do add credence to the possibility of a contribution from the corneal pathway.

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ACKNOWLEDGMENTS

This study was supported by Grant EY003297 from the Eye Institute, National Institutes of Health, and Allergan Pharmaceuticals, Irvine, Calif.

Factors Affecting Quinidine Protein Binding in Humans

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Abstract □ The free (unbound) concentration of drug in plasma is often an important determinant of pharmacological and toxicological effects. Unfortunately, studies examining the factors influencing the free fraction of quinidine in plasma have yielded inconsistent results. It is probable that differences in the type of blood collection tubes utilized and the analytical procedure employed biased some of these estimates of quinidine binding. The present study was executed in a manner free of factors now known to introduce artifacts into estimates of the free fraction of quinidine. In healthy volunteers, the free fraction of quinidine (1.0 μg/mL) was 0.129 ± 0.019 (mean ± SD) and was constant throughout the therapeutic range. A high-affinity, low-capacity binding site ($K = 1.17 \times 10^5$ M^{-1}; $nP = 3.49 \times 10^{-5}$ M) and a low-affinity, high-capacity binding site ($K = 1.33 \times 10^3$ M^{-1}; $nP = 3.14 \times 10^{-3}$ M) were identified. The characteristics of quinidine binding in a 4.5-g/dL solution of human serum albumin ($K = 3.05 \times 10^3$ M^{-1}; $nP = 1.36 \times 10^{-3}$ M) suggested that the low-affinity, high-capacity binding site was on this protein. In the presence of tris(butoxyethyl) phosphate (75 μg/mL), the quinidine free fraction increased from 0.114 to 0.231. A lidocaine concentration of 250 μg/mL caused a similar increase. Patients suffering traumatic injury had a significant increase in α_1-acid glycoprotein concentration (197 mg/dL) and a decreased quinidine free fraction (0.075 ± 0.019). Patients with hyperlipidemia had free fractions similar to those observed in healthy individuals (0.118 ± 0.019). These data suggest that the high-affinity, low-capacity binding site is on α_1-acid glycoprotein and that lipoproteins are of little importance in the protein binding of quinidine.

Keyphrases □ Protein binding—quinidine, humans □ Quinidine—protein binding, humans

Despite the recent development of a number of new agents, quinidine continues to be a mainstay of oral antiarrhythmic therapy. Effective concentrations of quinidine in serum range between 1 and 5 μg/mL (1, 2). However, much interindividual variability in the concentration necessary for arrhythmia suppression has been noted (2). Studies with other antiarrhythmic drugs, such as disopyramide (3) and propranolol (4), have demonstrated that the free drug concentration in plasma correlates better with the pharmacological effect than does the total concentration. These observations support the principle that free drug in plasma is in equilibrium with drug at receptor sites (*i.e.*, that which is responsible for the pharmacological effect). Furthermore, alterations in the degree of protein binding can also affect the pharmacokinetic properties of a drug. Fremstad *et al.* (5) have demonstrated a significant positive correlation between the free fraction of quinidine and both volume of distribution and total body clearance. For these reasons, a thorough understanding of the determinants of the protein binding of quinidine is important.

Unfortunately, studies in which the factors influencing quinidine protein binding have been examined have yielded inconsistent results. The mean free fraction in studies with healthy volunteers has been reported to be 0.10 (6), 0.105 (7), 0.141 (8), 0.246 (9), and 0.288 (10). Albumin, α_1-acid glycoprotein, and lipoproteins have all been reported to be important for quinidine binding, but their relative roles have been disputed (10–12). Some of the discrepancies between studies may have been due to methodological differences, as several factors have been found to influence quinidine binding *in vitro*. Quinidine free fraction is two- to three-fold higher when blood is collected in evacuated blood collection tubes[1] rather than glass syringes (6). Other factors influencing estimates of

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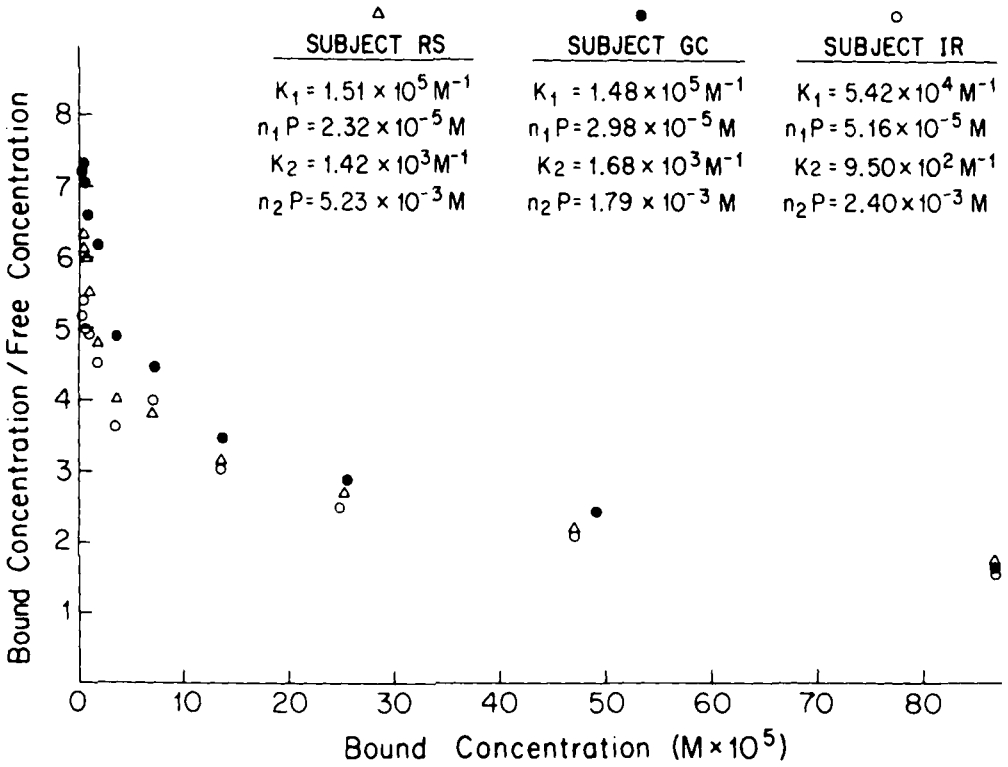

Figure 1—*Relationship between bound concentration/free concentration and bound concentration of quinidine in the serum of three healthy male volunteers.*

quinidine free fraction include the addition of heparin to blood *in vitro* (6), conditions under which equilibrium dialysis is performed (13), and the presence of dihydroquinidine, which is a common impurity in quinidine preparations (14). A principal purpose of the present investigation was to examine and clarify several aspects of quinidine protein binding in humans by using methods which do not bias estimates of the quinidine free fraction.

EXPERIMENTAL SECTION

Materials—Quinidine base was prepared from quinidine sulfate[2] by TLC (20% acetone in methanol) to separate quinidine from contaminants (primarily dihydroquinidine). [^{3}H]Quinidine[3] was also purified by this system, and a final purity of >98% was achieved.

Equilibrium Dialysis—Aliquots (400 μL) of phosphate buffer solution (pH 7.4, 0.134 M) containing quinidine (unlabeled, as well as a trace amount of radiolabeled drug) were dialyzed for 5 h at 37°C against an equal volume of serum in plexiglass cells. Postdialysis quinidine concentrations on each side of the dialysis membrane[4] were determined by liquid scintillation counting. All samples were assayed in triplicate, and the average coefficient of variation by this method was 2.97%. HPLC, coupled with liquid scintillation counting of the eluant, demonstrated that no degradation of quinidine occurred during the dialysis period.

Determination of Quinidine Binding Parameters: Rosenthal Analysis—Venous blood was obtained from three nonsmoking healthy male volunteers using glass syringes. The blood was transferred to glass tubes with polytef-lined screw caps and allowed to clot. The serum was removed after centrifugation and stored at 4°C. The serum was not frozen because of the possibility of structural alterations in lipoproteins (15). In all cases, binding studies were performed within 72 h of sample collection. The free fraction of quinidine was determined over a 2000-fold range of concentrations (initial quinidine concentrations, 0.5–1000 μg/mL), with the results plotted by the method of Rosenthal (16). The computer program PBNON[5] was used to estimate binding parameters. Quinidine binding in a solution of crystalline human serum albumin[6] (4.5 g/dL in phosphate buffer) was also determined over the same range of quinidine concentrations.

Effects of Potential Displacing Agents on Quinidine Binding—Serum obtained from a healthy volunteer was spiked with tris(butoxyethyl) phosphate[7] at a concentration of 75 μg/mL. Quinidine binding was studied over a range of concentrations from 0.5 to 1000 μg/mL, and the binding parameters obtained were compared with those from normal serum. Quinidine binding at a concentration of 1.0 μg/mL was also determined in serum containing lidocaine[8] at concentrations ranging from 1.0 to 500 μg/mL.

Effect of Hyperlipidemia and Trauma on the Serum Binding of Quinidine—Serum from six patients with elevated concentrations of lipoproteins and five healthy, nonsmoking male volunteers was obtained as described above and stored at 4°C for a maximum of 72 h before analysis. Quinidine binding was examined at an initial concentration of 1.0 μg/mL. In addition, quinidine binding was determined in two serum samples from each of four patients several days after traumatic injury (motor vehicle accidents). These samples were obtained as part of another investigation on the effect of trauma on α_1-acid glycoprotein concentration (17) and were handled in an identical manner to samples used in this investigation, with the exception that they were stored at −20°C. All samples were assayed for α_1-acid glycoprotein concentration by using a radial immunodiffusion procedure with commercially prepared kits[6]. Cholesterol and triglyceride concentrations were determined with an autoanalyzer by using the Liebermann–Burchard reagent and glycerol kinase method, respectively.

RESULTS

The quinidine serum protein binding data from three healthy volunteers are plotted (Fig. 1), by the method of Rosenthal (16). Two classes of binding sites are apparent over the concentration range studied. The affinity and capacity constants for each site are also shown (Fig. 1). At a concentration of 1.0 μg/mL, the free fraction of quinidine varied among individuals from 0.119 to 0.157 (mean, 0.138 ± 0.018). No evidence for clinically significant concentration-dependent changes in free fraction was observed within the usual therapeutic range (1–5 μg/mL). In isolated human serum albumin solution, quinidine binding was found to have characteristics consistent with those of the low-affinity, high-capacity class of sites in serum, with an observed value of 3.05×10^3 M^{-1} for the association constant (K) and 1.36×10^{-3} M for its binding capacity (nP; see Fig. 1).

[1] Vacutainer; Becton, Dickinson & Co.
[2] A. H. Robins Co., Richmond, Va.
[3] 100 mg of quinidine was supplied to New England Nuclear Corp., Boston, Mass. and was randomly tritiated by a catalytic exchange process (specific activity of the product, 0.24 mCi/mg).
[4] Spectrapor 2; Spectrum Medical Industries, Los Angeles, Calif.
[5] PBNON is a modification of the program MACMOL (written by Priore and Rosenthal in 1975) and generates protein binding parameters from the best nonlinear approximation of the data.
[6] Calbiochem-Behring, San Diego, Calif.
[7] Aldrich Chemical Co., Milwaukee, Wis.
[8] Astra Pharmaceutical Products, Inc., Worcester, Mass.

Table I—Mean Values for Quinidine Free Fraction and Concentration of Relevant Substances in Serum from Healthy Volunteers and Patients with Hyperlipidemia

	Mean ± *SD* Quinidine Free Fraction		
	Healthy Volunteers[a]	Hyperlipidemic Patients[b]	Statistical Significance[c]
Quinidine free fraction	0.129 ± 0.019	0.118 ± 0.019	NS[d]
Albumin, g/dL	4.8 ± 0.4	4.7 ± 0.3	NS
α_1-Acid glycoprotein, mg/dL	70 ± 16	99 ● 34	NS
Cholesterol, mg/dL	178 ± 14	313 ± 71	$p < 0.01$
Triglycerides, mg/dL	125 ± 32	245 ± 107[e]	$p < 0.05$

[a] $n = 5$. [b] $n = 6$. [c] t Test for independent samples. [d] Not significant. [e] The triglyceride value for one patient with a concentration of 2330 mg/dL was not included, as this would result in a badly skewed distribution of data.

Figure 2 contains the Rosenthal plot for quinidine binding in serum from a normal healthy male with and without the addition of tris(butoxyethyl) phosphate. The addition of this compound appeared to eliminate the high-affinity class of binding sites, resulting in a single class of sites with $K = 2.26 \times 10^3\ M^{-1}$ and $nP = 1.22 \times 10^{-3}$ M. In the presence of lidocaine, the quinidine free fraction increased with increasing lidocaine concentration, with a maximal value of 0.229 at a lidocaine concentration of 500 μg/mL[9]. This value is consistent with that observed at therapeutic quinidine concentrations in the presence of tris(butoxyethyl) phosphate (0.231) and with the predicted value; assuming the complete absence of the high-affinity class of binding sites in serum (0.272).

In the serum from patients suffering trauma, the mean α_1-acid glycoprotein concentration was 197 mg/dL (corresponding value in healthy volunteers, 70 mg/dL). The quinidine free fraction ranged from 0.049 to 0.113, with a mean value of 0.075 ± 0.019. The relationship between the quinidine binding ratio and the α_1-acid glycoprotein concentration is shown in Fig. 3. A strong correlation was observed, and the relationship was found to be quite consistent with that predicted by using the mean binding parameters shown in Fig. 1 (assuming that the high-affinity, low-capacity class of binding sites is on α_1-acid glycoprotein). Patients with hyperlipidemia had a mean cholesterol concentration of 313 mg/dL and a mean triglycerides concentration of 245 mg/dL (Table I). The free fraction of quinidine in these patients was 0.118 ± 0.019 and was not significantly different from the free fraction observed in healthy individuals (0.129 ± 0.019). In one patient with a triglyceride concentration of 2330 mg/dL, the quinidine free fraction was 0.128.

DISCUSSION

The protein binding of quinidine has been the subject of a number of previous investigations, many of which have yielded conflicting results. The recent identification of a number of factors which may artificially alter estimates of the quinidine free fraction prompted the present investigation, with the goal of obtaining definitive and clinically useful data.

The mean free fraction of quinidine in healthy volunteers has varied widely among studies (range, 0.10–0.29). The value of 0.129 observed in this investigation is near the lower limit of previously reported values. Two main classes of binding sites were identified in the serum from healthy volunteers, with both sites contributing nearly equally to the fraction of drug bound at therapeutic concentrations of quinidine. Because of this, one would predict that the free fraction would only double in the complete absence of one or the other of these classes of sites.

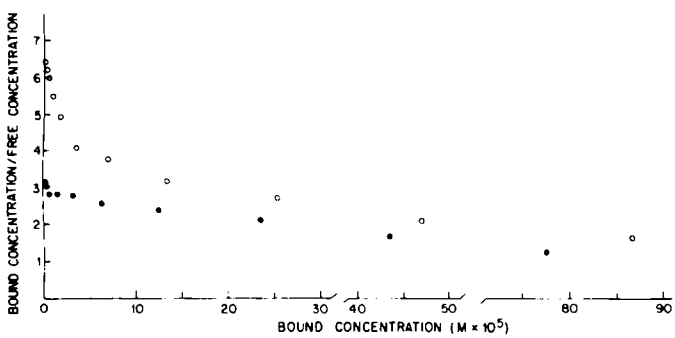

Figure 2—*Relationship between bound concentration/free concentration and bound concentration of quinidine in serum from a healthy male volunteer (○) and identical serum, to which tris(butoxyethyl) phosphate (●) at 75 μg/mL was added.*

Knowledge of the identity and characteristics of the binding proteins allows the prediction of the consequences of pathophysiological or pharmacological changes in the binding milieu. In this investigation, the binding of quinidine in an isolated albumin solution was found to have characteristics similar to the binding of quinidine to the low-affinity class of binding sites in serum. However, previous investigations have suggested that α_1-acid glycoprotein and lipoproteins, as well as albumin, contribute significantly to quinidine binding (11, 12). In attempting to establish the relative contributions of these proteins, quinidine binding was studied in the presence of tris(butoxyethyl) phosphate, a compound known to displace basic drugs from α_1-acid glycoprotein binding sites (18). The quinidine free fraction increased in a manner that was consistent with the absence of binding to the high-affinity class of sites. Because little is known about the specificity of tris(butoxyethyl) phosphate protein binding (*i.e.*, it could possibly also displace quinidine from lipoproteins), lidocaine was also studied as a potential displacer of quinidine. Lidocaine is known to be bound primarily to α_1-acid glycoprotein, with some binding to albumin and no demonstrable affinity for lipoproteins (17, 19). The ability of lidocaine to displace quinidine to the same extent as tris(butoxyethyl) phosphate suggests that interactions between quinidine and lipoproteins do not influence the free fraction of this drug.

The results of the quinidine binding studies in the trauma patients and hyperlipidemic patients support our conclusions concerning the identity of the binding sites. Trauma patients have been shown to have high concentrations of α_1-acid glycoprotein and increased binding of lidocaine (17). In this study, quinidine free fraction was reduced by >40% at a mean α_1-acid glycoprotein concentration of 197 mg/dL. Since concentrations of this magnitude are observed in several common conditions (acute myocardial infarction, rheumatoid arthritis, and cancer), similarly reduced quinidine free fractions are likely in many patients receiving the drug (20). A reduced free fraction has been reported in surgical patients (21) and in survivors of cardiac arrest (22). Using computer simulations, Kates *et al.* (10) have predicted that elevated concentrations of lipoproteins would have little effect on the quinidine binding ratio. Our results support this conclusion, as the small difference in

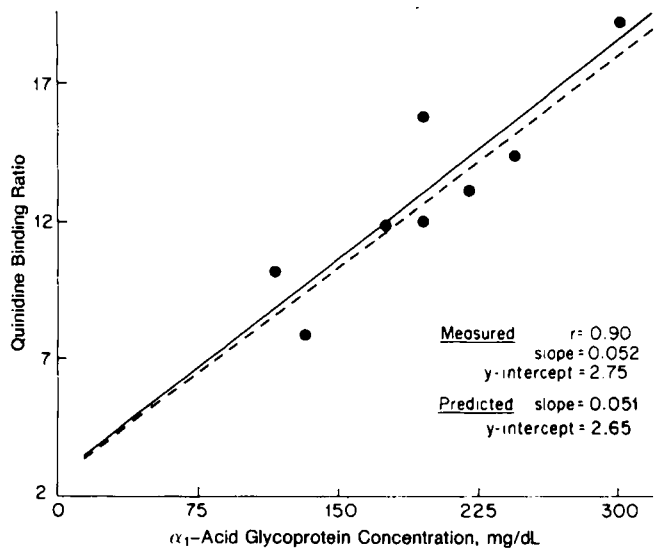

Figure 3—*Relationship between quinidine binding ratio (bound concentration/free concentration) and α_1-acid glycoprotein concentration in serum obtained from patients after traumatic injury. Predicted relationship (- - -) was obtained with the mean constants from Fig. 1 and by assuming site 1 to be on α_1-acid glycoprotein; (——) the best-fit line by linear regression analysis (measured).*

[9] Unpublished results.

free fraction between the hyperlipidemic patients and the healthy volunteers can be accounted for by slightly higher α_1-acid glycoprotein concentrations in the former group.

It appears, therefore, that the important binding proteins for quinidine are α_1-acid glycoprotein and albumin. Using the mean binding parameters for these proteins (Fig. 1), one can predict that at least a 50% decrease in albumin concentration would be necessary to obtain a 20% increase in quinidine free fraction. Clinically significant changes in quinidine binding are more likely to be due to changes in α_1-acid glycoprotein concentration, since serum levels of this protein may be increased in patients suffering from a number of common diseases.

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ACKNOWLEDGMENTS

This study was supported in part by Grant 65-0566 from the British Columbia Heart Foundation and by Public Health Service Grant GM-20852 from the Institute of General Medical Sciences, National Institutes of Health.

Simple, Rapid Method for Comparing the Self-Emulsifiability of Hydrocarbon Oils

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Received October 25, 1982, from the **Department of Pharmaceutics, University of Ife, Ile-Ife, Nigeria* and the ‡*Department of Pharmacy, Chelsea College, University of London, Manresa Road, London SW3, U.K.* Accepted for publication May 12, 1983.

Abstract □ A simple and rapid method for comparing the degree of self-emulsifiability of different hydrocarbon oils is described. The method involved measurement of the intensity of light scattered at an angle of 31° to the incident radiation by the sample. The extent or degree of self-emulsification of selected hydrocarbon oils was observed to be affected by the nature of the oil as well as by the type and concentration of the surfactants employed. The method is useful when screening surfactant–hydrocarbon oil combinations as potential vehicles for drugs in the pharmaceutical industry or herbicides and pesticides for agricultural purposes.

Keyphrases □ Emulsions—surfactant-hydrocarbon oil, self-emulsifiability, measurement by laser nephelometry □ Laser nephelometry—self-emulsifiability, surfactant-hydrocarbon oil mixtures □ Hydrocarbon oils—self-emulsifiability, effect of added surfactants, measurement by laser nephelometry

Self-emulsifiable oils (also known as emulsifiable concentrates), widely used in the chemical and allied industries because they readily form emulsions without the need for powerful or sophisticated emulsification equipment, serve as vehicles for herbicides and pesticides and are used for cutting and rolling metals into thin sheets. They are also employed as lubricants in the textile industry and are currently being used for the recovery and processing of crude oils.

Self-emulsifiable oils have some potential applications in the drug industry. Solutions of drugs in oils have been administered to patients in soft gelatin capsules since the early part of the 19th century. In its modern form, this dosage is claimed to be advantageous since the accuracy, stability, and patient convenience is greater than that for the corresponding tableted form of a given drug. These oily solutions readily emulsify when released into the aqueous environment of the stomach. The generation of a large surface area means an optimum condition for extraction and absorption of the drug. The main requirement would be a suitable combination of nontoxic surfactants in bland oils.

It has been reported that the oil droplets in self-emulsifiable systems can be very small, ~1 μm (1). Measurement of droplet sizes in the submicrometer range has proved to be very tedious and/or time consuming (2). It is anticipated that the laser nephelometer[1], when used judiciously, can reduce the number of problems associated with particle size characterization in dispersed systems.

THEORETICAL SECTION

Several theories of light scattering by small particles (3–5) have appeared in the literature since the pioneering work of Rayleigh (6, 7) in this field. Recently, Bagchi and Vold (8) have commented on the limitations of some of these theories. In the present work, it is assumed that the basic equation

[1] Hyland Laser Nephelometer PDQ; Travenol Laboratories.

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