J. Pharm. Pharmacol. 1991, 43: 650–654
Received September 12, 1990

The Influence of α_1-Acid Glycoprotein on Quinine and Quinidine Disposition in the Rat Isolated Perfused Liver Preparation

S. M. MANSOR*, S. A. WARD†, G. EDWARDS*†, P. E. HOAKSEY* AND A. M. BRECKENRIDGE*

** Department of Pharmacology and Therapeutics, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK, and † Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK*

Abstract—We have studied the effect of 0·5 and 2·0 g L^{-1} of α_1-acid glycoprotein (AAG) on the disposition of quinine and quinidine in the rat isolated perfused liver preparation. The higher concentration of AAG (2·0 g L^{-1}) resulted in a significant decrease in clearance [quinine study (control: 9·6 ± 2·9 vs test: 3·1 ± 1·2 mL min^{-1}); quinidine study (control: 9·8 ± 2·4 vs test: 3·5 ± 1·1 mL min^{-1})] and volume of distribution [quinine study (control: 1198 ± 416 vs test: 466 ± 95 mL); quinidine study (control: 1352 ± 459 vs test: 317 ± 24 mL)] but not the elimination half-life compared with control. At the lower concentration (0·5 g L^{-1}) of AAG there was no significant difference in clearance, volume of distribution and elimination half-life for either drug compared with control. By increasing the concentration of AAG from 0·5 to 2·0 g L^{-1} both the hepatic extraction ratio and the fraction of drug unbound when compared with controls significantly decreased by about 66 and 60% for quinine, and by 65 and 58% for its diastereoisomer quinidine, respectively. The consequence of these changes is a substantial increase in the total quinine (or quinidine) concentrations without any change in the free quinine (or quinidine) concentrations. However, at 0·5 g L^{-1} AAG compared with control, no significant difference was observed in fraction of drug unbound, extraction ratio, total drug concentration or free drug concentration for either drug. In summary, changing concentrations of AAG, an important binding protein for quinine and quinidine, can affect the hepatic disposition of both drugs.

α_1-Acid glycoprotein (AAG) is known to increase non-specifically in response to certain pathophysiological changes (Kremer et al 1988). During malaria, plasma AAG is elevated (Voulgari et al 1982) with a compensatory fall in albumin (Silamut et al 1985). AAG is an important plasma constituent that binds basic drugs such as quinine and quinidine (Piafsky 1980; Pike et al 1981). Both of these drugs bind to AAG rather than albumin in-vitro (Mihaly et al 1987a) and so changes in AAG concentrations occasioned by malaria infection may influence the disposition of quinine and quinidine. In this paper, we describe the effects of circulating protein profiles, namely AAG on the pharmacokinetics of these two drugs in the rat isolated perfused liver preparation. This experimental model has been used successfully to study the effect of protein binding on the hepatic clearance of a number of drugs (Schary & Rowland 1983; Smallwood et al 1988).

Materials and Methods

Study design

Male Wistar rats, 200–250 g, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and their livers isolated using standard procedures and then perfused in a constant flow (15 mL min^{-1}) recirculating system at 37°C as previously described (Mansor et al 1990a). The principal indices of liver viability were steady oxygen consumption over 4 h (1·5–2·0 μmol (g liver)$^{-1}$ min^{-1}), sustained bile flow (0·4–0·6 mL h^{-1}), constant perfusion pressure (8–12 cm H_2O), reproducible liver function tests and normal visual appearance. The livers were divided into three groups; (a) control, without the addition of bovine AAG (n = 6) to the perfusate (Mansor et al 1990a); (b) addition of 0·5 g L^{-1} of AAG (Sigma, Poole, UK) (n = 4) [the normal physiological concentration (Putnam 1975)]; and (c) addition of 2·0 g L^{-1} of AAG (n = 4) [the concentration observed during malaria infection (Voulgari et al 1982)].

Quinine dihydrochloride or quinidine hydrochloride monohydrate (Sigma, Poole, UK) was introduced into the perfusate reservoir of the three groups as an aqueous solution (0·1 mL; 10 mM). The experiment lasted for 4 h. Samples of perfusate (1 mL) were removed predose and at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min and perfusate plasma removed following centrifugation (13 000 rev min^{-1}). Additional samples (1 mL) were also removed at 60, 120, 180 and 240 min for the measurement of protein binding of both drugs by ultrafiltration, as previously described (Mansor et al 1990b). After the removal of each sample, an equal volume of drug-free perfusate was added to the reservoir. Bile was collected hourly during each experiment and stable bile flow was maintained by constant infusion of sodium taurocholate (30 μmol h^{-1}; 0·5 mL h^{-1}; Sigma, Poole, UK). Perfusate plasma and bile were frozen and stored at −20°C until analysis by HPLC.

Drug analysis and sample preparation

The concentration of quinine or quinidine in samples of perfusate was estimated by a selective and sensitive HPLC procedure adapted from Mihaly et al (1987b) and reported previously (Mansor et al 1990a).

Correspondence and present address: S. A. Ward, Department of Pharmacology and Therapeutics, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK.

Data analysis

Data in text and tables are presented as mean ± s.d. and graphically as mean ± s.e.m. Perfusate plasma elimination half-life ($t_{\frac{1}{2}}$) was calculated as 0·693/k where k is the elimination rate constant obtained by regression analysis of the log linear portion of the perfusate plasma concentration vs time curve. Area under the curve (AUC_{0-t}) values were obtained by linear trapezoidal summation and $AUC_{t-\infty}$ was calculated from C_t/k where C_t is the concentration at time t. $AUC_{0-\infty}$ was then determined by summation of these values. Other pharmacokinetic parameters (total hepatic clearance (CL), volume of distribution (V_d)) were calculated using standard model-independent formulae (Rowland & Tozer 1989). The following parameters were calculated using equations as described by Pang & Rowland (1977). $C_u = f_u C_t$ where C_u is the free concentration of quinine or quinidine, fu is the fraction of drug unbound in perfusate plasma at t = 0 and C_t is the total perfusate plasma concentration. Intrinsic clearance of unbound drug (CL'_{int}) was calculated from the equation below

$$CL'_{int} = \frac{QE}{f_u(1-E)}$$

where E is the hepatic extraction ratio, Q is liver blood flow and CL'_{int} is intrinsic clearance of unbound drug. E was determined using CL/Q. Statistical comparisons were made using one-way analysis of variance rejecting the null hypothesis where $P < 0{\cdot}05$.

Results

Liver viability

In this study, the principal indices of liver viability were not significantly different amongst the three groups as evidenced by consistency in the values for the liver function tests (sodium, potassium, alkaline phosphatase, alanine aminotransferase, aspartate transaminase and γ-glutamyl transaminase), oxygen consumption at the end of perfusion (quinine [control: 1·9 ± 0·5 vs test: 1·8 ± 0·6 μmol (g liver)$^{-1}$ min^{-1}]; quinidine [control: 1·9 ± 0·4 vs test: 2·0 ± 0·6 μmol (g liver)$^{-1}$ min^{-1}) and final perfusion pressure quinine (control: 9·8 ± 2·1 vs test: 10·0 = 1·8 cm H_2O); quinidine (control: 10·5 ± 1·9 vs test: 11·0 ± 1·6 cm H_2O) and bile flow (quinine (control: 0·42 ± 0·15 vs test: 0·48 ± 0·11 mL h^{-1}); quinidine (control: 0·39 ± 0·13 vs test: 0·40 ± 0·14 mL h^{-1})).

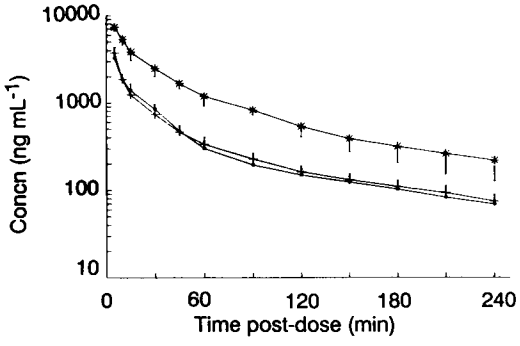

FIG. 1. Mean perfusate concentration-time profiles for quinine in three groups of rat isolated perfused preparations. Control, ●; 50 mg/100 mL AAG, +; 200 mg/100 mL AAG, *.

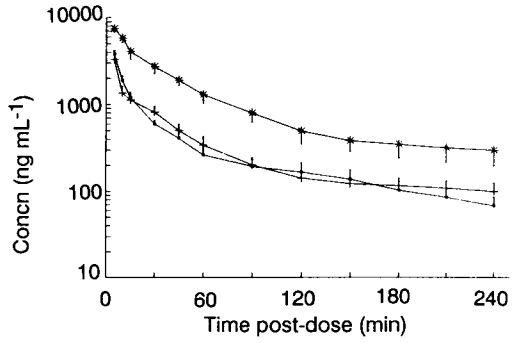

FIG. 2. Mean perfusate concentration-time profiles for quinidine in three groups of rat isolated perfused liver preparations. Control, ●; 50 mg/100 mL AAG, +; 200 mg/100 mL AAG, *.

Perfusate disposition of quinine and quinidine

The mean (± s.e.m.) perfusate concentration-time profiles for quinine and quinidine are shown in Figs 1 and 2. The perfusate plasma concentration of both drugs in all groups appeared to decay biexponentially. Tables 1 and 2 list mean (s.d.) pharmacokinetic parameters for quinine and quinidine, respectively.

Effect of AAG on protein binding

The percentage of unbound quinine and quinidine in all the three groups of perfused livers is shown in Figs 3 and 4, respectively. The binding of quinine to bovine albumin and AAG was similar to that of quinidine in all experiments. The fraction of unbound drug as compared with control is substantially smaller ($P < 0{\cdot}001$) in groups of livers where 2·0 g L^{-1} AAG was added at the start of the experiments. There was no significant difference in fraction of unbound drug between 0·5 g L^{-1} AAG group and the control group. For both drugs, the fraction of drug unbound increased in all groups over the four hour study.

Discussion

Clinical studies suggest that the pharmacokinetics of quinine (White et al 1982), the drug of choice for the treatment of severe and complicated chloroquine-resistant falciparum malaria (World Health Organization 1986) and its diastereoisomer quinidine (Phillips et al 1985) are altered during malaria. Factors such as impairment of hepatic drug metabolism (Trenholme et al 1976), changes in circulating plasma proteins and, as a consequence, altered drug binding (Silamut et al 1985), particularly AAG (Voulgari et al 1982) and changes in hepatic blood flow (Molyneux et al 1989) may each contribute to the alteration in the pharmacokinetics observed. Since quinine and quinidine have narrow therapeutic indices, the higher plasma concentrations of these drugs observed during malaria as a result of changes in their pharmacokinetics have given rise to concern. However, in the clinical situation, the three factors operate concurrently and thus complicate the interpretation of the pharmacokinetic changes. This warranted separate investigations of each potential variable in suitable animal models in order to understand their individual roles in the pharmacokinetic alterations seen when these drugs are used clinically. In this

Table 1. Pharmacokinetic parameters for quinine in the rat isolated perfused liver preparation.

Parameter	Control	50 mg/100 mL AAG	200 mg/100 mL AAG
AUC (ng h mL^{-1})	1856±460	2006±659	6035±2374*
Clearance (CL) ($mL\ min^{-1}$)	9·57±2·87	8·89±2·38	3·10±1·17*
Volume of distribution (Vd) (mL)	1198±416	1690±330	466±95*
Half-life ($t_{\frac{1}{2}}$) (h)	1·47±0·39	2·27±0·44	1·88±0·64
Hepatic extraction (E)	0·67±0·2	0·62±0·18	0·21±0·08*
Clearance intrinsic unbound (CL'_{int}) ($mL\ min^{-1}$)	49·8±34·9	54·3±26·0	26·5±6·0
Percentage of drug unbound			
1 h	50·8±6·6	51·0±3·7	20·3±4·6*
4 h	66·3±4·5	64·8±5·9	33·3±3·5*
Total concentration (C_t) ($ng\ mL^{-1}$)			
1 h	263±125	342±179	1129±175*
4 h	70±44	101±49	301±202*
Free concentration (C_u) ($ng\ mL^{-1}$)			
1 h	140±60	170±78	223±30
4 h	44±27	63±26	95±55

* $P<0·01$, ** $P<0·001$, ANOVA, compared with control.

Table 2. Pharmacokinetic parameters for quinidine in the rat isolated perfused liver preparation.

Parameter	Control	50 mg/100 mL AAG	200 mg/100 mL AAG
AUC (ng h mL^{-1})	1798±492	1861±504	5311±2143**
Clearance (CL) ($mL\ min^{-1}$)	9·80±2·38	9·47±2·51	3·45±1·05**
Volume of distribution (Vd) (mL)	1352±459	1309±685	317±24*
Half-life ($t_{\frac{1}{2}}$) (h)	1·59±0·42	1·57±0·58	1·19±0·55
Hepatic extraction (E)	0·65±0·16	0·63±0·17	0·23±0·07**
Clearance intrinsic unbound (CL'_{int}) ($mL\ min^{-1}$)	60·8±27·2	60·3±29·5	28·8±3·4
Percentage of drug unbound			
1 h	52·7±4·6	53·3±5·9	22·0±4·2**
4 h	68·8±5·8	67·0±5·0	35·3±5·9**
Total concentration (C_t) ($ng\ mL^{-1}$)			
1 h	302±126	336±138	970±101*
4 h	69±20	75±27	189±124*
Free concentration (C_u) ($ng\ mL^{-1}$)			
1 h	155±52	173±53	213±45
4 h	47±10	49±15	65±36

* $P<0·01$, ** $P<0·001$, ANOVA, compared with control.

study, the animal model chosen is sufficiently sensitive to demonstrate the effect of changes in circulating AAG concentrations on quinidine and quinine disposition throughout the physiological range. Our results demonstrate that changes in the concentration of AAG can alter significantly the pharmacokinetic characteristics of both drugs in the rat isolated perfused liver preparation. Plasma protein binding of these two cinchona alkaloids has previously been investigated, although the effect of altered concentrations of AAG, an important binding protein for quinine and quinidine on their pharmacokinetics has yet to be explored.

The present studies show that at physiological concentrations of AAG (0·5 g L^{-1}, group b) there was no significant difference in total hepatic CL, Vd and $t_{\frac{1}{2}}$ for either drug compared with control. In our study, the concentration of albumin used in all three groups was 1 g/100 mL which is 20-fold greater than the AAG levels of group b (albumin: 1 g/100 mL and AAG: 50 mg/100 mL). In addition, at physiological concentrations, both drugs bind to albumin (60–75%) in-vitro (Nilsen 1976; Mihaly et al 1987a). Therefore, at the protein composition in control and group b, binding of either drug to proteins will not be greatly altered (Figs 3, 4) by

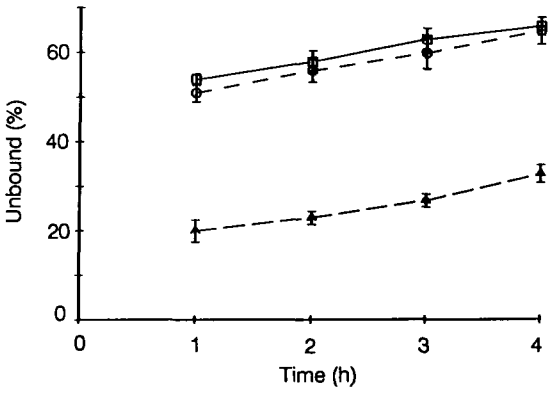

FIG. 3. Mean percentage of quinine unbound in the perfusate time in three groups of rat isolated perfused liver preparations. Control □, 50 mg AAG ○, 200 mg AAG ▲.

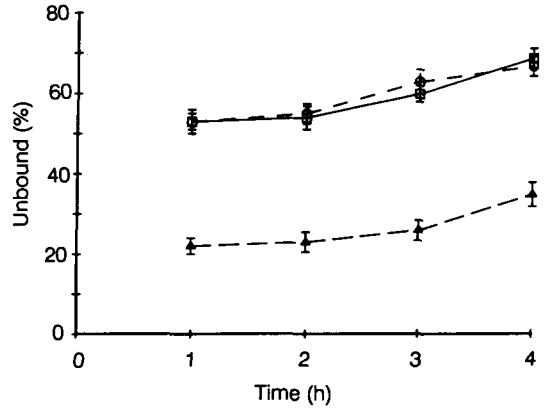

FIG. 4. Mean percentage of quinidine unbound in the perfusate time in three groups of rat isolated perfused liver preparations. Control □, 50 mg AAG ○, 200 mg AAG ▲.

addition of AAG. Hence there is no change in the fraction unbound (f_u) observed for either drug (Tables 1, 2). The hepatic extraction ratio (E) determined in control and group b is about 0·6–0·7 for both quinine and quinidine indicating that these two cinchona alkaloids exhibit an intermediate extraction ratio (Wilkinson & Shand 1975) and, in man, quinidine is classified as having an intermediate extraction ratio (Rowland & Tozer 1989). Addition of 0·5 g L^{-1} AAG did not alter the unbound concentration or the extraction ratio of either drug compared with control and hence there was no change in the disposition parameters of quinine or quinidine (Tables 1, 2).

In contrast, at 2·0 g L^{-1} AAG (group c) simulating the concentration observed during malaria infection, both total hepatic CL and V_d of quinine and quinidine were reduced significantly without any change in $t_{\frac{1}{2}}$ compared with group b and control. In this case a four fold increase in AAG concentrations resulted in a threefold decrease in unbound drug concentrations and their respective extraction ratios for quinine and quinidine. As a consequence the extraction characteristics of both drugs are reduced from intermediate (0·3–0·7) to a low hepatic extraction compound ($E < 0·3$). Altering unbound drug concentrations over a wide range of protein concentrations is possible since both drugs exhibit protein concentration-dependent binding (Mihaly et al 1987a). Therefore, reducing the extraction ratio from intermediate to low by altering the fraction of the drug unbound results in a significant decrease in total hepatic CL and increased total drug concentrations but not the free concentration or the intrinsic CL of unbound drug (Tables 1, 2). This type of change in disposition parameters is well documented for drugs having low extraction ratios, i.e. restrictively eliminated drugs (Wilkinson & Shand 1975).

Interestingly, the fraction of quinine or quinidine unbound increased over time in all three groups (Figs 3, 4). This phenomenon could result from the irreversible loss of bovine AAG from the perfusion circuit due to the foreign properties of bovine AAG compared with rat. It has been reported that there are specific differences with respect to the physical and chemical properties of rat AAG compared with that from bovine and human sources (Binette 1968; Yoshima et al 1981). However, this cannot explain the gradual increase in f_u in the control rat livers where AAG was omitted. It is possible that drug metabolites or waste products of liver function may compete with these drugs for binding sites. However, there is no information available to support or contradict this view. Additionally, alterations in perfusate pH as a result of CO_2 production may be expected to alter drug binding (Christensen et al 1983); however, in these studies perfusate pH was continually monitored and buffered to 7·4 with saturated $NaHCO_3$. It is therefore difficult to explain these observations although comparable time-related alterations were observed in all studies.

These data indicate that changes in AAG can affect the disposition of quinine and quinidine in the rat isolated perfused liver preparation suggesting that plasma protein binding is an important determinant in drug disposition. This would be important for those drugs which are highly bound, where factors which influence plasma proteins quantitatively will influence the various disposition parameters, notably Vd and CL. This highlights the need to monitor free drug concentrations, disease-induced changes in AAG levels and their relationship to the pharmacological and toxicological response especially for drugs such as quinidine and quinine with narrow therapeutic indices.

Acknowledgement

S. M. Mansor is supported by the Academic Staff Training Scheme of the National Drug Research Centre, Universiti Sains Malaysia. S. A. Ward is a Wolfson Lecturer.

References

Binette, J. P. (1968) Pattern of polymorphism of alpha1-acid glycoprotein in different species. Biochim. Biophys. Acta 154: 234–235

Christensen, J. H., Andreasen, F., Jensen, E. B. (1983) The binding of thiopental to human serum albumin at variable pH and temperature. Acta. Pharmacol. Toxicol. 52: 364–370

Kremer, J. M. H., Wilting, J., Janssen, L. H. M. (1988) Drug binding to human alpha$_1$-acid glycoprotein in health and disease. Pharmacol. Rev. 40: 1–47

Mansor, S. M., Ward, S. A., Edwards, G., Hoaksey, P. E., Breckenridge, A. M. (1990a) The effect of malaria infection on the disposition of quinine and quinidine in the rat isolated perfused liver preparation. J. Pharm. Pharmacol. 42: 428–432

Mansor, S. M., Taylor, T. E., McGrath, C. S., Edwards, G., Ward, S. A., Wirima, J. J., Molyneux, M. E. (1990b) Intramuscular

quinine in Malawian children with moderately severe falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. 84: 482–487

Mihaly, G. W., Ching, M. S., Kleijn, M. B., Paull, J., Smallwood, R. A. (1987a) Differences in the binding of quinine and quinidine to plasma proteins. Br. J. Clin. Pharmacol. 24: 769–774

Mihaly, G. W., Hayman, K. M., Smallwood, R. A. (1987b). High performance liquid chromatographic analysis of quinine and its diastereoisomer quinidine. J. Chromatogr. 415: 177–182

Molyneux, M. E., Looareesuwan, S., Menzies, I. S., Grainger, S. L., Phillips, R. E., Wattanagoon, Y., Thompson, R. P. H., Warrell, D. A. (1989) Reduced hepatic blood flow and intestinal malabsorption in severe falciparum malaria. Am. J. Trop. Med. Hyg. 40: 470–476

Nilsen, O. G. (1976) Serum albumin and lipoproteins as the quinidine binding molecules in normal human sera. Biochem. Pharmacol. 25: 1007–1012

Pang, K. S., Rowland, M. (1977) Hepatic clearance of drug. I. Consideration of a "well-stirred" model and a "parallel tube" model. Influence of hepatic blood flow, plasma and blood cell binding and the hepatocellular enzymatic activity on hepatic drug clearance. J. Pharmacokinet. Biopharm. 5: 625–653

Phillips, R. E., Warrell, D. A., White, N. J., Looareesuwan, S., Karbwang, J. (1985) Intravenous quinidine for the treatment of severe falciparum malaria. Clinical and pharmacokinetic studies. New Eng. J. Med. 312: 1273–1278

Piafsky, K. M. (1980) Disease-induced changes in the plasma binding of basic drugs. Clin. Pharmacokinet. 5: 246–262

Pike, E., Skuterud, B., Kierulf, P., Fremstad, D., Abdel Sayed, S. M., Lunde, P. K. M. (1981) Binding and displacement of basic, acidic and neutral drugs in normal and orosomucoid-deficient plasma. Ibid. 6: 367–374

Putnam, F. W. (1975) Alpha, beta, gamma, omega. The roster of the plasma proteins. In: Putnam, F. W. (ed.) The Plasma Proteins. Academic Press London. Vol. I pp 58–125

Rowland, M., Tozer, N. T. (1989) Clinical Pharmacokinetics. Concepts and Applications. Lea and Febiger, London

Schary, W. L., Rowland, M. (1983) Protein binding and hepatic clearance: studies with tolbutamide, a drug of low intrinsic clearance, in the isolated perfused rat liver preparation. J. Pharmacokinet. Biopharm. 11: 225–243

Silamut, K., White, N. J., Looareesuwan, S., Warrell, D. A. (1985) Binding of quinine to plasma proteins in falciparum malaria. Am. J. Trop. Med. Hyg. 34: 681–686

Smallwood, R. H., Mihaly, G. W., Smallwood, R. A., Morgan, D. J. (1988) Effect of protein binding change on unbound and total plasma concentrations for drugs of intermediate hepatic extraction. J. Pharmacokinet. Biopharm. 16: 529–542

Trenholme, G. M., Williams, R. L., Rieckman, K. H., Frischer, H., Carson, P. E. (1976) Quinine disposition during malaria and during induced fever. Clin. Pharmacol. Ther. 19: 459–467

Voulgari, F., Cummins, P., Gardecki, T. I. M., Beeching, N. J., Stone, P. C., Stuart, J. (1982) Serum levels of acute phase and cardiac proteins after myocardial infarction, surgery and infection. Br. Heart. J. 48: 352–356

White, N. J., Looareesuwan, S., Warrell, D. A., Warrell, M. J., Bunnag, D., Harinasuta, T. (1982) Quinine pharmacokinetics and toxicity in cerebral and uncomplicated falciparum malaria. Am. J. Med. 73: 564–572

Wilkinson, G. R., Shand, D. G. (1975) A physiological approach to hepatic drug clearance. Clin. Pharmacol. Ther. 18: 377–390

World Health Organization (1986) Severe and complicated malaria. Trans. R. Soc. Trop. Med. Hyg. 80: 1–50

Yoshima, H., Matsumoto, A., Mizuochi, T., Kawasaki, T., Kobata, A. (1981) Comparative study of the carbohydrate moeities of rat and human plasma alpha1-acid glycoproteins. J. Biol. Chem. 256: 8476–8484