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Age-dependent Alteration of the Serum-unbound Fraction of Nicardipine, a Calcium-channel Blocker, in Man

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Abstract

To determine whether the age-dependent increase in the pharmacological effect of calcium-channel blockers is a result of age-dependent alteration of the unbound fraction the drug in serum, the unbound fraction of the nicardipine was investigated in the serum of 38 adults.

The unbound concentration of nicardipine in serum to which nicardipine (205·4 ng mL^{-1}) had been added was determined by ultracentrifugation to range from 0·49 to 4·01% (mean ± s.d., 1·55 ± 0·78%). Non-glycosylated albumin was most strongly correlated with age ($r = 0·901$). Total bilirubin was weakly correlated with age whereas levels of α-1-acid glycoprotein, triglycerides and glycosylated albumin were not correlated with age. A significant ($P < 0·01$) linear correlation was obtained between the unbound fraction of nicardipine and parameters such as age, albumin, albumin/globulin ratio, albumin/glycosylated albumin ratio, non-glycosylated albumin and total bilirubin. To assess the relative effect of each variable on the unbound fraction of nicardipine, stepwise multiple linear regression was performed using age and biochemical parameters. The three variables (non-glycosylated albumin, total bilirubin and age) were entered into the regression equation.

The results of this study showed that the major ligand of nicardipine in serum was non-glycosylated albumin, which decreased with age. It was, moreover, shown that the serum-unbound concentration of nicardipine increased with age. This finding would be one factor accounting for the increase in the pharmacological effect of nicardipine with age. In addition, our predicted model for the unbound fraction of nicardipine might be useful in determining the appropriate nicardipine dose for the elderly.

Serum-unbound fraction is an important determinand in drug pharmacokinetics, especially for drugs which have strong binding characteristics. As the unbound concentration increases, a greater pharmacological effect can be expected. Alteration of the unbound fraction of the drug will also influence various pharmacokinetic parameters, notably the apparent volume of distribution and clearance.

The most important factors which can influence serum protein profiles are ageing and the disease process. Grainger-Rousseau (1989) has stated in a review that some disease states influence the binding of drugs to plasma proteins. Common pathological conditions that lead to an altered serum-unbound fraction are cardiac, renal and hepatic disease, etc. The change in the unbound fraction was mainly based on the alteration of the serum albumin level caused by these disease states. Serum albumin levels, moreover, obviously decrease with age (Castleden & George 1979). On the other hand, α-1-acid glycoprotein is also an important drug-binding protein for basic and neutral drugs, although albumin has a greater binding capacity. The α-1-acid glycoprotein level can vary as a result of certain diseases (Kremer et al 1988) and increase with age (Verbeeck et al 1984). Despite these considerations modification of the individual dosing interval and amount has not been substantially performed in clinical use with elderly patients.

Calcium-channel blockers are being advocated for use in the treatment of geriatric patients with hypertension. These drugs bind strongly to serum proteins. It has been reported that the pharmacological effect of calcium-channel blockers such as verapamil, etc., increase with increasing age (Abernethy et al 1986; Buhler et al 1982) and it is presumed that the age-dependent response is caused by alteration of pharmacokinetic factors (for example, changing metabolic ability). It is, on the other hand, also assumed that the age-dependent response is caused by an increase in the sensitivity and affinity of receptors for the drugs, namely, an increase in pharmacodynamic response (Abernethy et al 1986), although this mechanism has not been substantiated and investigation of alteration of the unbound fraction of this drug has not been reported. The determination of the serum-unbound concentration is difficult because these drugs bind strongly to serum proteins, and the range of serum concentration for common oral medications is very low. There is, therefore, little published information on the protein binding of calcium-channel blockers.

Nicardipine hydrochloride, a calcium-channel blocker which binds strongly to serum protein, is widely used in the treatment of essential hypertension and the improvement of cerebral circulation. This paper describes the age-dependent alteration of the unbound fraction of nicardipine in serum.

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Materials and Methods

Chemicals and reagents

Nicardipine was kindly supplied by Yamanouti Pharmaceutical Co. Ltd (Tokyo, Japan). All other chemicals were of reagent grade.

Subjects

This investigation was approved by the Health Authority Ethics Committee in the Kyoto Prefectural University of Medicine.

Serum from 38 adults (22 male, 16 female), 22 to 93 years, was obtained after an overnight fast. Venous blood (12 mL) was collected into glass centrifuge tubes. Samples were left to clot for 60 min at room temperature and were then centrifuged at 3000 rev min^{-1} for 10 min to obtain the serum fraction (5 mL). To measure the biochemical parameters, 2 mL of serum was stored at −20°C before analysis. The measured biochemical parameters were: total protein, albumin, α-1-acid glycoprotein, glycosylated albumin, albumin/glycosylated albumin ratio, albumin/globulin ratio, triglycerides, total bilirubin, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, creatinine and urea nitrogen. For the protein binding study, 3 mL serum was used within 24 h of collection of the blood.

Binding study of nicardipine

The unbound concentration of nicardipine in serum was determined by an ultracentrifugation method. The nicardipine solution (0·1 mM) was prepared in methanol and this solution (12 μL) was added to serum (3 mL; total concentration of nicardipine, 205·4 ng mL^{-1}, methanol concentration, 0·4% (it has been reported (Monks et al 1978) that a methanol concentration of 0·4% in serum is permissible for binding studies). After incubation at 37°C for 1 h, the mixtures were centrifuged at 250 000 *g* for 20 h. The centrifuge was left to stop without braking to minimize turbulence. The supernatant was used to determine the unbound drug. The concentration of protein remaining in the supernatant was determined by the method of Markwell et al (1978). To check whether nicardipine was adsorbed by the ultracentrifuge tube nicardipine (205·4 ng mL^{-1}) in 0·01 M phosphate buffer (pH 7·4) was incubated at 37°C for 1 h in a centrifuge tube and the amount of nicardipine was measured incubation.

The amount of nicardipine in the supernatant obtained by ultracentrifugation (the unbound concentration of nicardipine) was determined by GC with an electron capture detector (Higuchi & Shiobara 1980b). Samples were prepared by liquid-liquid extraction with toluene. Nicardipine analysis was performed on single samples because it was difficult to obtain sufficient sample volume. The detection limit of this assay method was 1·0 ng mL^{-1} from a 1-mL sample. The inter- and intra-assay reproducibilities (c.v.) of the method were, respectively, 8·9 and 7·3% (1·0 ng mL^{-1}, n = 10). There is no problem in determining the nicardipine level, because the lowest concentration of nicardipine in the unbound fraction observed in this experiment was 2·0 ng mL^{-1}.

Statistical analysis

The relationship between variables was examined by single and multiple linear regression. A P value less than 0·01 was considered as indicative of statistical significance.

Results

Table 1 shows the basic statistics of the biochemical parameters and the serum-unbound fraction of nicardipine in the subject population (n = 38). The ranges of glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, creatinine and urea nitrogen of all samples were normal. The correlation matrix for age and parameters such as total protein, albumin, glycosylated albumin, albumin/glycosylated albumin ratio, α-1-acid glycoprotein, albumin/globulin ratio, triglycerides and total bilirubin for all samples was examined. The correlation between age and non-glycosylated albumin, calculated by subtracting glycosylated albumin levels from albumin levels, was also examined (Table 2). Albumin, total protein, non-glycosylated albumin and albumin/globulin ratio levels decreased significantly with age, whereas albumin/glycosylated albumin ratio increased with age. Fig. 1 shows the relationship between non-glycosylated albumin and age. Non-glycosylated albumin was most strongly correlated with age (r = 0·901). Total bilirubin was weakly correlated with age. Alpha-1-acid glycoprotein, triglycerides and glycosylated albumin levels were not correlated with age. There was, on the other hand, no correlation between albumin and glycosylated albumin.

No protein was detected in the supernatant obtained by ultracentrifugation (the unbound fraction of nicardipine) even though the detection limit of protein in this study was 5 μg mL^{-1} and the protein concentration of the serum was about 64–84 mg mL^{-1}. In addition, no adsorption of nicardipine by the ultracentrifuge tubes was detected. The unbound fraction of nicardipine ranged from 0·49 to 4·01% (mean ± s.d. 1·55 ± 0·78%) relative to a total nicardipine concentration of 205·4 ng mL^{-1}. As shown in Table 2, a significant linear correlation was obtained between the unbound fraction of nicardipine and parameters such as age, albumin, albumin/globulin ratio, albumin/glycosylated albumin ratio, non-glycosylated

Table 1. Basic statistics of the biochemical parameters, age and the unbound fraction of nicardipine.

Parameter	Mean	s.d.	Minimum	Maximum
Age (year)	63	24	22	93
Unbound fraction of nicardipine (%)	1·55	0·78	0·49	4·01
Total protein (g dL^{-1})	6·9	0·62	5·5	8·3
Albumin (g dL^{-1})	4·3	0·56	3	5·3
Glycosylated albumin (%)	15·0	2·30	11·1	19·2
α-1-Acid glycoprotein (mg dL^{-1})	84·3	29·8	40·2	177
Albumin to globulin ratio	1·69	0·38	1·04	2·64
Total bilirubin (mg dL^{-1})	0·67	0·36	0·25	2·2
Triglycerides (mg dL^{-1})	114	59·6	38	243
Glutamic oxaloacetic transaminase (IU L^{-1})	20	6·1	13	47
Glutamic pyruvic transaminase (IU L^{-1})	15	7·9	8	46
Creatinine (mg dL^{-1})	0·75	0·14	0·4	1·0
Urea nitrogen (mg dL^{-1})	16	4·4	10	25

Table 2. Correlation matrix for the biochemical parameters, age and the unbound fraction of nicardipine.

	Correlation coefficient, r	
	Age	Unbound fraction of nicardipine
Age (year)	—	—
Unbound fraction of nicardipine (%)	0·493*	—
Total protein (g dL^{-1})	−0·680‡	−0·376
Albumin (g dL^{-1})	−0·867‡	−0·568‡
Glycosylated albumin (%)	0·670‡	0·488*
α-1-Acid glycoprotein (mg dL^{-1})	0·276	−0·038
Albumin to globulin ratio	−0·775‡	−0·531†
Total bilirubin (mg dL^{-1})	−0·496*	0·413*
Triglycerides (mg dL^{-1})	−0·105	0·190
Non-glycosylated albumin	−0·901‡	−0·596‡

*$P < 0·01$, †$P < 0·001$, ‡$P < 0·0001$

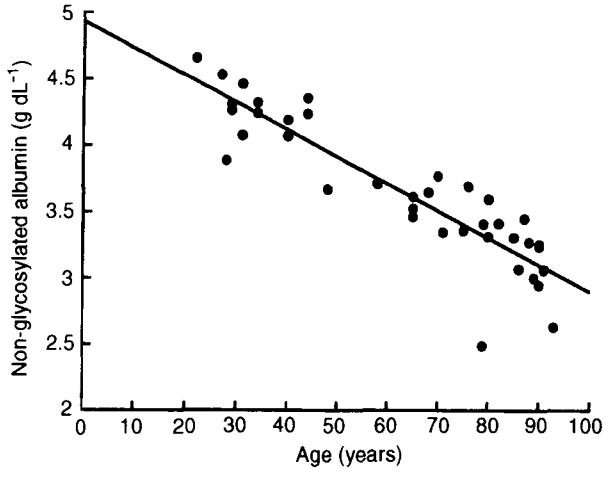

FIG. 1. Correlation with age of the concentration of non-glycosylated albumin in the serum from 38 subjects.

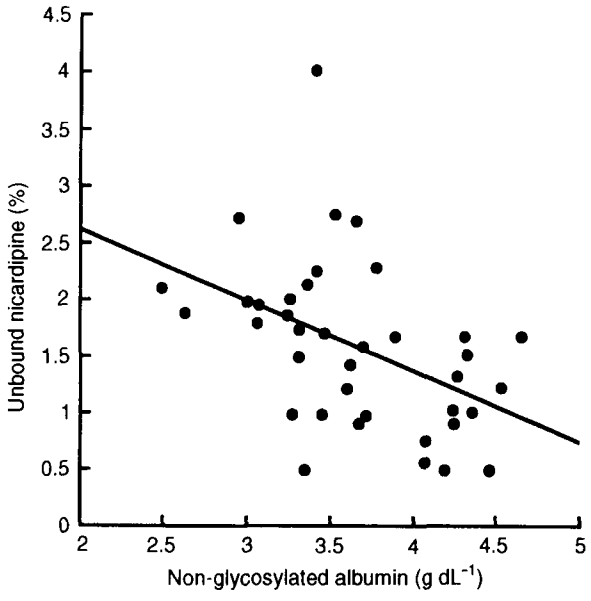

FIG. 2. Correlation of nicardipine unbound fraction with the concentration of non-glycosylated albumin in the serum from 38 subjects.

albumin or total bilirubin. Non-glycosylated albumin was strongly correlated with the unbound fraction of nicardipine ($r = 0·596$, $P < 0·0001$). Fig. 2 shows the relationship between the unbound fraction of nicardipine and non-glycosylated albumin. Age was also correlated with the unbound fraction of nicardipine ($r = 0·493$, $P < 0·001$). The relationship between the unbound fraction of nicardipine and age is shown in Fig. 3. Total bilirubin was weakly correlated with the unbound fraction of nicardipine ($r = 0·413$, $P < 0·01$) whereas total protein, α-1-acid glycoprotein, glycosylated albumin and triglycerides were not.

To assess the relative effect of each variable on the unbound fraction of nicardipine, stepwise multiple linear regression was performed using age and biochemical parameters except albumin, albumin/globulin ratio, glycosylated albumin, albumin/glycosylated albumin ratio and total protein, because non-glycosylated albumin reflects these five parameters. The three variables (non-glycosylated albumin, total bilirubin and age) were selected and entered in the regression equation (Table 3). Adding more variables (α-1-acid glycoprotein, triglycerides, etc.) did not result in a significant ($P < 0·01$) increase in the multiple correlation coefficient. The statistical significance for the regression of this predictive model was $P < 0·0005$ (F-test). The correlation coefficient for actual and predicted values was 0·938 (passed through the origin, $P < 0·0001$), suggesting the possibility of clinical usefulness of this model.

Discussion

Previous studies suggest that calcium blockers might be more effective in older than younger patients, as was described in the introduction. There are several postulated mechanisms for this, e.g. increased sensitivity and affinity of receptors for the drug and the alteration of pharmacokinetic factors, a low renin state (McDonald et al 1978) and a reduction in baroreceptor reflex sensitivity (Gribbin et al 1979) in elderly patients with essential hypertension. The result of our present study could offer a new postulate for the mechanism by which a calcium blocker might be more effective in older than younger patients. The range of the serum-unbound fraction of nicardipine was 0·49–4·01% in this study. This change in the unbound con-

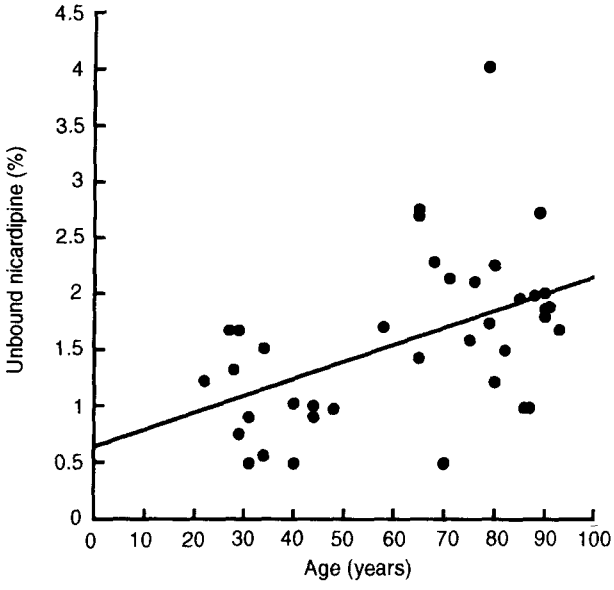

FIG. 3. Correlation with age of the unbound nicardipine fraction from 38 subjects.

centration, the important parameter, shows a remarkable influence on the pharmacological effects and toxicity of nicardipine. In addition, it has been reported that the hepatic extraction ratio was high (Drici et al 1993; Ahmed et al 1991). The total clearance of nicardipine depends, therefore, on the hepatic blood flow, which decreases with age, rather than serum-protein binding ratio.

Nicardipine is rapidly absorbed after oral administration. It has been reported that the maximum concentration of nicardipine in plasma after an single oral dose of 10-40 mg was 13–253 ng mL^{-1}, values which were observed at 0·5–1 h (Higuchi & Shiobara 1980a). It has also been reported that the plasma concentration of nicardipine in patients with cerebrovascular disease who received oral medication at a daily dose of 60 mg was 58-219 ng mL^{-1} (Higuchi et al 1980). The concentration of nicardipine in the sample of our binding study, 205·4 ng mL^{-1}, thus reflects the clinical situation.

In the study of the binding of drugs in serum, equilibrium dialysis, ultrafiltration and ultracentrifugation are generally used for the determination of the unbound drug concentration (Whitlam & Brown 1981; Broers et al 1983; Barre et al 1985). Because a preliminary experiment showed that the degree of adsorption of nicardipine on to the membranes used in ultrafiltration and equilibrium dialysis devices was very high, we adopted the ultracentrifugation method. Nicardipine did not bind to the ultracentrifugation tubes.

It is well known that serum protein concentrations change with age. A decrease in serum albumin levels and an increase in serum globulin concentrations with age has been generally described (Cammarata et al 1967; Castleden & George 1979; Verbeeck et al 1984). In our present investigation, a decrease in albumin level was observed (from 5·3 g dL^{-1} at 22 years to 3·0 g/100 mL at 93 years). An age-dependent elevation of serum α-1-acid glycoprotein levels has, however, also been reported (Kremer et al 1988). In general, the α-1-acid glycoprotein level is increased by inflammation. Although there is a high possibility that elderly patients will have an inflammatory disease, the subjects in this study were essentially healthy. This might explain why α-1-acid glycoprotein was not correlated with age in our study.

Albumin is considered to be the major binding protein for most acidic drugs. Many basic drugs generally exhibit only moderate affinity for albumin and bind to other serum proteins such as α-1-acid glycoprotein and lipoproteins (Piafsky 1980). The unbound fraction of nicardipine, a basic drug, was not, however, correlated with the α-1-acid glycoprotein level in our present study, and the unbound fraction of nicardipine was strongly correlated with the albumin level. Although basic drugs such as disopyramide show great affinity for α-1-acid glycoprotein (Kremer et al 1988), the serum-unbound fraction of the basic drug desipramine is not correlated with α-1-acid glycoprotein levels but shows a correlation with albumin levels (Verbeeck et al 1984). These characteristics might explain why albumin has a greater binding capacity than α-1-acid glycoprotein and that the affinity of both nicardipine and desipramine for α-1-acid glycoprotein is relatively lower than that of other basic drugs.

Chronic hyperglycaemia results in non-enzymatic glycosylation of many proteins, such as haemoglobin, albumin, etc. Glycosylated albumin has been used as an index of glycaemic control in diabetics (Kemp et al 1984). It has been reported that the unbound fraction of drugs is related to the extent of albumin glycosylation in serum (Ruiz-Cabello and Erill 1984). Kemp et al (1987) reported that the unbound fraction of phenytoin correlated well with non-glycosylated albumin. In our present study the best correlation was also found between the unbound fraction of nicardipine and non-glycosylated albumin (Fig. 2). Because we also found that non-glycosylated albumin was well correlated with age (Fig. 1), the increase in the unbound fraction of nicardipine with age as shown in Fig. 3 might be a consequence of the decrease in non-glycosylated albumin with age.

The other biochemical parameters, such as bilirubin, free fatty acid, etc., might, on the other hand, have an effect on the unbound fraction of nicardipine. In our present study, total bilirubin was also correlated with age and the unbound fraction

Table 3. Multiple linear regression model for the unbound fraction of nicardipine.

Dependent variable	Selected independent variables	Coefficient
Unbound fraction of nicardipine (%)	Age (year)	−0·011
	Non-glycosylated albumin (g dL^{-1})	−1·174
	Total bilirubin (mg dL^{-1})	−0·485
	Intercept	6·871

$P < 0·0005$, F-test.

of nicardipine. Stepwise multiple linear regression analysis identified three parameters, age, non-glycosylated albumin and total bilirubin, as the explanatory variables to the serum-unbound fraction of nicardipine. The serum-unbound fraction is an important factor of the distribution volume and clearance. These parameters are needed for the individual pharmacokinetic modelling of the drug's serum profile after oral dosing. It is, however, as described in the introduction, difficult to determine the unbound serum concentration of nicardipine. This model for predicting the unbound fraction of nicardipine on the basis of age, non-glycosylated albumin and total bilirubin might, therefore, be useful for modification of the individual dosing of elderly patients. In addition, because non-glycosylated albumin was the most effective predictive factor for estimating the unbound fraction of nicardipine, we are now investigating altered binding of nicardipine in diabetic patients.

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