J. Pharm. Pharmacol. 1999, 51: 1009–1014
Received December 14, 1998
Accepted March 12, 1999

Age-related Alteration of Carbamazepine–serum Protein Binding in Man

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Abstract

To determine whether biological maturation influences the kinetics of carbamazepine-serum protein binding, the carbamazepine free fraction (%) was investigated in the serum of 66 patients, ranging from 4 to 83 years, with epilepsy or trigeminal neuralgia, treated with carbamazepine alone or carbamazepine in combination with phenytoin, phenobarbital, and/or valproic acid, over a relatively long period.

Biochemical parameters such as levels of albumin and non-glycated albumin showed a significant relationship with carbamazepine free fraction ($r = -0{\cdot}521$, $P < 0{\cdot}001$ for albumin; $r = -0{\cdot}700$, $P < 0{\cdot}001$ for non-glycated albumin). Non-glycated albumin was more strongly correlated with carbamazepine free fraction. The biochemical parameters showed a significant relationship with age ($r = -0{\cdot}243$, $P < 0{\cdot}1$ for albumin; $r = 0{\cdot}666$, $P < 0{\cdot}001$ for glycated albumin; $r = -0{\cdot}459$, $P < 0{\cdot}001$ for non-glycated albumin; $r = 0{\cdot}640$, $P < 0{\cdot}001$ for carbamazepine free fraction). Glycated albumin (%), non-glycated albumin and carbamazepine free fraction (%) were strongly correlated with age, whereas albumin showed only a weak correlation with age. To evaluate the effects of ageing on carbamazepine–serum protein binding, the patients were divided into three groups according to age: children, 4–15 years; adults, 16–64 years; elderly, 65–83 years. Albumin and non-glycated albumin were much lower, and glycated albumin (%) and carbamazepine free fraction (%) much higher in the elderly group than in the other two groups.

The results of this study showed that the major ligand of carbamazepine in the serum was non-glycated albumin, which decreased with age. These observations suggested that in elderly patients, the elevation of free carbamazepine concentrations in the serum caused by reduced non-glycated albumin levels, induces increases in the sensitivity of the pharmacological effects of carbamazepine and the risk of drug interactions.

The serum-free fraction is an important determinant in drug pharmacokinetics, in particular for drugs having strong binding characteristics. As the free concentration increases, greater pharmacological effects can be expected. Alteration of the free fraction of the drug will also affect various pharmacokinetic parameters, such as the apparent volume of distribution and clearance. The most important factors which influence the free fraction in the serum are changes in the levels of binding proteins such as albumin and α_1-acid glycoprotein in the serum, and a competitive displacement between the drug and other ligands at binding sites on protein molecules. Alteration of albumin levels is known to be associated with disease states such as cardiac, renal and hepatic diseases. Greenblatt (1979) reported that serum albumin levels decreased with age. Several authors have demonstrated that changes in binding disposition of serum protein for drugs are important in determining the variable free fraction; for example albumin is non-enzymatically modified by glucose in the blood (glycation) (Stevens et al 1978; Monnier et al 1979; McVerry et al 1980; Chiou et al 1981; Gonen et al 1981; Otsuka & Kadis 1981; Pape et al 1981; Schleicher et al 1981; Zaman & Verwilghen 1981; Kohn & Schnider 1982; Kemp et al 1984, 1987;

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Doucet et al 1993), which alters not only the binding ability of albumin, but also its binding capacity for drugs.

Carbamazepine has been used extensively in patients with complex and generalized seizures, trigeminal neuralgia and bipolar affective disorders. Dosages have been adjusted by monitoring serum carbamazepine concentrations because carbamazepine has a narrow therapeutic range, and pharmacologically adverse effects are related to serum carbamazepine concentrations. Carbamazepine binds strongly to serum proteins, approximately 75% in the therapeutic range (Hooper et al 1975; Lawless et al 1982; MacKichan & Zola 1984), and mainly to serum albumin (Kodama et al 1993). Changes in albumin levels and its binding characteristics, therefore, contribute greatly to the variable carbamazepine free fraction (%), and alter the free concentrations in the serum, which may affect patients receiving carbamazepine.

Biological maturation has complex and poorly understood effects on drug disposition, but there have been no reports concerning the influences of maturational changes on carbamazepine–serum protein binding. In this study, we describe age-related alterations of biochemical parameters such as albumin levels and glycated albumin (%), and the effects of ageing on carbamazepine–serum protein binding.

Materials and Methods

Reagents and chemicals

Carbamazepine, fatty acid-free human serum albumin (HSA), and glycated human serum albumin (glycated-HSA), which has 1–5 mol hexose/mol albumin, were purchased from Sigma (St Louis, MO). KH_2PO_4 and Na_2HPO_4 were obtained from Nacalai Tesque (Kyoto, Japan).

In-vitro binding study

Correlation between carbamazepine free fraction (%) and glycated-HSA (%). After adjusting the HSA or glycated HSA concentration to 600 μM by addition of phosphate buffer (pH 7·4), these were mixed with glycated HSA at 0, 30, 60 and 90%. The molecular weights of HSA and glycated HSA were assumed to be 66 400 and 66 800, respectively (Okabe & Hokaze 1993). Carbamazepine 10 $\mu g\,mL^{-1}$ was added to the albumin mixtures. Following incubation for 60 min at 37°C in a shaking water bath, total and free carbamazepine concentrations were measured in each sample. Total and free carbamazepine concentrations were determined as described in the in-vivo binding study section. To assess the influence of glycation of albumin on carbamazepine–albumin binding, simple linear regression analysis was performed using carbamazepine free fraction (%) and glycated HSA (%).

In-vivo binding study

Subjects. The investigation was approved by the Health Authority Ethics Committee of Kyoto Prefectural University of Medicine.

Serum samples used in this study were obtained from 66 patients with epilepsy or neuralgia, treated with carbamazepine alone or carbamazepine in combination with phenytoin, phenobarbital, and/or valproic acid, over a relatively long period. In all the patients, autoinductive effects were considered to be complete and a steady state was assumed to have been achieved. The patients' ages ranged from 4 to 83 years (mean age ± s.d., 24·8 ± 20·8 years). Patients with diabetes mellitus were excluded from this study. Venous blood 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. To measure the biochemical parameters, 2 mL serum was stored at −20°C before analysis. The biochemical parameters measured were: albumin, α_1-acid glycoprotein, glycated albumin (%), total bilirubin and free fatty acids. Glycated albumin measurement was performed by Kyoto Biseibutsu Kenkyusyo (Kyoto, Japan). For the protein binding study, 1 mL serum was used within 24 h of collection of the blood.

Assay of the serum concentrations of total and free carbamazepine. Total carbamazepine concentrations in the serum from patients were assayed using standard fluorescence immunopolarization TDx (Dainabot) kits. The same serum samples were centrifuged at 1500 rev min^{-1} for 5 min using a micropartition system starter kit (MPS-1) (Amicon Div., W.R. Grace & Co. Danvers, MA) with Diaflo ultrafiltration membranes (YMT-membrane) (Amicon Div., W.R. Grace & Co. Danvers, MA). After ultrafiltration, the filtrates containing free carbamazepine were assayed with TDx. All experiments were performed in duplicate. The adsorption rate (%) for the YMT-membrane was evaluated at two carbamazepine saline concentrations, 5 and 10 $\mu g\,mL^{-1}$, before and after ultrafiltration according to the following equation:

$$\text{Adsorption (\%)} = ((\text{Total carbamazepine concn} - \text{Free carbamazepine concn})/\text{Total carbamazepine concn}) \times 100$$

Evaluation of various factors influencing carbamazepine–serum protein binding. We investigated the relationship between carbamazepine free fraction (%) and the biochemical parameters, and also the effects of ageing on these parameters.

To evaluate the effects of glycation on carbamazepine–serum protein binding, the patients were divided into two groups according to glycated albumin (%): normal group, 10–15; high group, 15 and over. The values for these groups, such as non-glycated albumin levels and carbamazepine free fraction (%), were compared. Non-glycated albumin levels were calculated from the albumin level and glycated albumin (%). To evaluate the effects of ageing on glycation and carbamazepine–serum protein binding, the patients were divided into three groups according to age: children, 4–15 years; adults, 16–64 years; elderly, 65–83 years. The values of glycated albumin (%) and carbamazepine free fraction (%), and the levels of albumin and non-glycated albumin in each group were compared.

Results

The adsorption rates (%) for the YMT-membrane were $7{\cdot}2 \pm 1{\cdot}5$ and $5{\cdot}5 \pm 2{\cdot}5$% at 5 and 10 $\mu g\,mL^{-1}$ carbamazepine, respectively. Both adsorption rates were lower than 10%.

The in-vitro binding study showed that carbamazepine free fraction (%) was strongly correlated to glycated HSA (%); carbamazepine free fraction (%) = 0·14·glycated HSA (%) + 23·11.

The correlation coefficient was 0·992 ($P < 0{\cdot}001$).

In the in-vivo binding study, the serum levels ($g\,dL^{-1}$) (mean value ± s.d.) of albumin and non-glycated albumin were $4{\cdot}3 \pm 0{\cdot}5$ and $3{\cdot}8 \pm 0{\cdot}4$, respectively, and glycated albumin (%) and carbamazepine free fraction (%) were $13{\cdot}9 \pm 2{\cdot}2$ and $25{\cdot}8 \pm 3{\cdot}2$, respectively, in the subject population (n = 66). The mean serum α_1-acid glycoprotein level ($mg\,dL^{-1}$) was $71{\cdot}6 \pm 14{\cdot}9$. The levels of α_1-acid glycoprotein showed little interpatient variation, ranging from 55 to 99, which was within the normal range (50–100). The mean serum total bilirubin and free fatty acid levels were $0{\cdot}344 \pm 0{\cdot}100$ ($mg\,dL^{-1}$) and 311 ± 229 ($\mu eq\,L^{-1}$).

The relationships between carbamazepine free fraction (%) and albumin or non-glycated albumin are shown in Figures 1 and 2 , respectively. These showed a significant relationship with carbamazepine free fraction (%): $r = -0{\cdot}521$, $P < 0{\cdot}001$ for albumin; $r = -0{\cdot}700$, $P < 0{\cdot}001$ for non-glycated albumin. Non-glycated albumin was more strongly correlated with carbamazepine free fraction (%). α_1-Acid glycoprotein, total bilirubin and free fatty acid levels showed no significant correlations with carbamazepine free fraction (%).

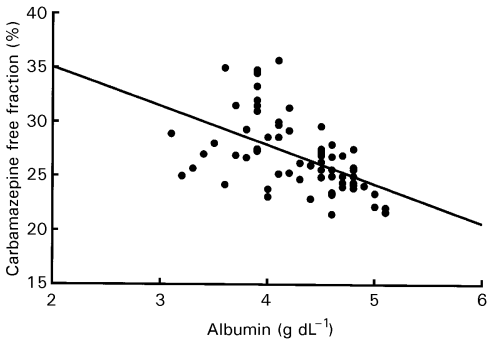

Figure 1. Correlation of carbamazepine free fraction (%) with albumin levels in the serum in 66 patients.

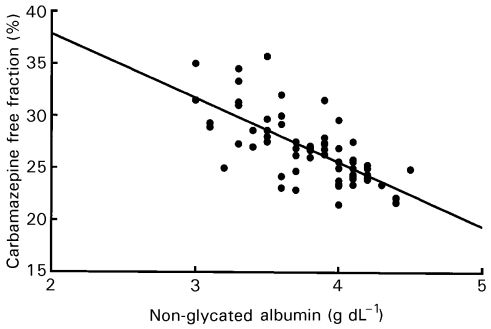

Figure 2. Correlation of carbamazepine free fraction (%) with non-glycated albumin levels in the serum in 66 patients.

The relationships between age and non-glycated albumin or carbamazepine free fraction (%) are shown in Figures 3 and 4, respectively. These parameters showed significant correlations with age: $r = -0{\cdot}459$, $P < 0{\cdot}001$ for non-glycated albumin; $r = 0{\cdot}640$, $P < 0{\cdot}001$ for carbamazepine free fraction (%). In addition, albumin and glycated albumin (%) were correlated with age: $r = -0{\cdot}243$, $P < 0{\cdot}1$; $r = 0{\cdot}666$, $P < 0{\cdot}001$, respectively. Non-glycated albumin, carbamazepine free fraction (%), and glycated albumin (%) were highly correlated with age, whereas albumin was weakly correlated with age. α_1-Acid glycoprotein, total bilirubin and free fatty acid levels showed no significant correlations with age.

Table 1 summarizes albumin, non-glycated albumin levels and carbamazepine free fraction (%) in the two groups, with normal and high glycated albumin (%). There was no difference in the albumin level, whereas there were significant differences in both the non-glycated albumin level and carbamazepine free fraction (%) between the groups. The high glycated albumin (%) group had decreased non-glycated albumin levels, and increased carbamazepine free fraction (%) compared with the normal group.

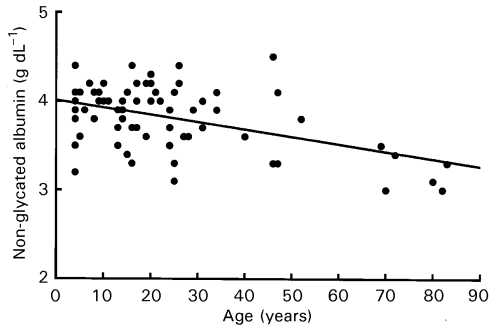

Figure 3. Correlation of non-glycated albumin levels in the serum with age.

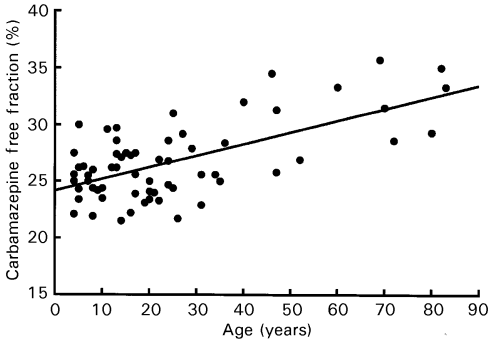

Figure 4. Correlation of carbamazepine free fraction (%) with age.

Table 2 shows the biochemical parameters and carbamazepine free fraction (%) in the child, adult and elderly groups. There were significant differences in all biochemical parameters examined except total bilirubin, α_1-acid glycoprotein and free fatty acids between the elderly group and the other groups. Albumin and non-glycated albumin levels were much lower, and glycated albumin (%) and carbamazepine free fraction (%) were much higher in the elderly group than in the other two groups. The adult group had slightly higher glycated albumin (%) compared with the child group, whereas there were no differences in the other biochemical parameters between these two groups.

To assess the relative effect of each variable on carbamazepine free fraction (%), stepwise multiple linear regression analysis was performed using age and biochemical parameters. The two variables (non-glycated albumin and age) were selected, and entered in the regression equation (Table 3).

Table 1. Effect of glycation on carbamazepine–serum protein binding.

Group	Glycated albumin (%)	n	Albumin (g dL^{-1})	Non-glycated albumin (g dL^{-1})	Carbamazepine free fraction (%)
Normal	10–15	43	4·5 ± 0·3	4·0 ± 0·2	25·5 ± 2·4
High	≥15	23	4·3 ± 0·4	3·6 ± 0·4*	29·1 ± 3·9*

Values are mean ± s.d. *$P < 0{\cdot}001$ compared with the normal group.

Table 3. Multiple linear regression model for the carbamazepine free fraction.

Dependent variable	Selected independent variable	Coefficient
Carbamazepine free fraction (%)	Age (years)	0·046
	Non-glycated albumin (g dL^{-1})	−5·284
	Intercept	45·452

$P < 0{\cdot}005$, F-test.

Discussion

Previous studies (Hooper et al 1975; Lawless et al 1982) revealed that there is a large interpatient variability in serum carbamazepine free fraction (%). Hooper et al (1975) studied the largest population (74 patients), and found a 4-fold variation between individuals with a mean carbamazepine free fraction (%) of 26·9%, and a range of 8·5–34·3%. In addition, Lawless et al (1982) also reported a 5-fold variation between individuals, with a mean carbamazepine free fraction (%) of 24·9% and a range of 10·0–47·8%. Our results were in accordance with those reports. Serum albumin levels in individuals in this study were within the normal range, and there was little interpatient variation, whereas carbamazepine free fraction (%) varied greatly, ranging from 21·5 to 35·7. With regard to this variation, Contin et al (1985) reported

Table 2. Effects of biological maturation on carbamazepine–serum protein binding.

Group	Age	n	Glycated albumin (%)	Albumin (g dL^{-1})	Non-glycated albumin (g dL^{-1})	Total bilirubin (mg dL^{-1})	Free fatty acids (μeq L^{-1})	α_1-Acid glycoprotein (mg dL^{-1})	Carbamazepine free fraction (%)
Child	4–15	26	13·1 ± 1·1	4·5 ± 0·3	3·9 ± 0·2	0·330 ± 0·113	360 ± 131	71 ± 13	25·7 ± 2·3
Adult	16–64	33	14·2 ± 2·2*	4·5 ± 0·3	3·9 ± 0·3	0·341 ± 0·083	239 ± 144	65 ± 10	26·7 ± 3·5
Elderly	65–83	7	17·3 ± 1·9†	4·0 ± 0·4†	3·3 ± 0·3†	0·387 ± 0·124	386 ± 480	77 ± 15	31·1 ± 4·0‡

Values are mean ± s.d. *$P < 0{\cdot}05$, compared with the child group. †$P < 0{\cdot}001$, compared with the other groups. ‡Significantly different from the child group ($P < 0{\cdot}001$) and the adult group ($P < 0{\cdot}005$).

the influence of variable serum α_1-acid glycoprotein levels, and observed a progressive decrease in carbamazepine free fraction (%) as serum α_1-acid glycoprotein increased. Serum α_1-acid glycoprotein levels have been reported to be elevated in inflammation, myocardial infarction, cancer and trauma (Piafsky & Borga 1977; Paxton 1983), and moreover in elderly subjects (Greenblatt 1979; Wallace & Verbeeck 1987). In this study, however, no significant correlation was observed between α_1-acid glycoprotein level and carbamazepine free fraction (%). Serum α_1-acid glycoprotein levels in individuals in this study were within the normal range as none of the patients suffered from the diseases described above, although there were small interpatient variables. In this study, serum α_1-acid glycoprotein levels did not affect carbamazepine–serum protein binding. Albumin is therefore considered to be a major binding protein for carbamazepine and we focused on the effects of altered albumin levels on carbamazepine binding.

Serum albumin is capable of reversible binding of drug molecules to its surface and of functioning as a buffer and transporter in drug distribution and elimination processes. Serum concentrations of free drugs which exhibit pharmacological effects are influenced by changes in albumin levels and its binding affinity for drugs. The amount of glycated serum proteins is known to be higher in patients with diabetes mellitus than in healthy controls. Several authors (Ruiz-Cabello & Erill 1984; Kemp et al 1987; McNamara et al 1988; Doucet et al 1993) reported that serum protein binding of drugs was decreased in diabetics. Kemp et al (1987) reported that there was no significant correlation between the phenytoin free fraction (%) and glycated albumin level, whereas the non-glycated albumin level was strongly correlated with the phenytoin free fraction (%). Our observation that an increase in carbamazepine free fraction (%) was related to a reduction in non-glycated albumin level was in agreement with their findings, and suggested that non-glycated albumin is the main binding protein for carbamazepine in serum. These findings suggested that the risk of adverse effects of drugs which bind strongly to albumin is elevated in patients with hypoalbuminaemia or diabetes mellitus. On the other hand, free fatty acids and bilirubin also affect the binding of drugs such as diazepam and sulfisoxazole to serum proteins (Ruiz-Cabello & Erill 1984). Our results, however, indicated that these factors show no significant correlation with carbamazepine free fraction (%), suggesting that carbamazepine molecules bind to different sites on the albumin molecules.

The elderly group showed significant differences in glycated albumin (%), albumin, non-glycated albumin levels and carbamazepine free fraction (%) compared with the other groups (Table 2). Moreover, stepwise multiple linear regression analysis identified two parameters, age and non-glycated albumin, as the explanatory variables to the carbamazepine free fraction (%) (Table 3). These results suggested that with ageing, the progression of the glycation of serum proteins and the reduced albumin levels co-operatively decreased non-glycated albumin levels, which greatly affected the carbamazepine free fraction (%). Despite the lack of patients with diabetes mellitus in this study, glycated albumin (%) was higher in the elderly patients than in the other groups. This was thought to have been because elderly subjects had almost equal amounts of glycated albumin but lower total albumin levels compared with the other groups. In addition, it is well known that the synthesis and metabolism of endogenous substances are physiologically decreased in elderly subjects. This decreased metabolism might allow albumin to remain in the serum for a relatively long time, and prolong the exposure of the remains to glucose in the serum, resulting in the increased glycated albumin (%) observed in the elderly.

Carbamazepine is highly bound to serum protein and has a low hepatic extraction ratio (Yerby et al 1985). The total clearance of carbamazepine depends, therefore, on hepatic metabolism. The elevated carbamazepine free fraction (%) induces increases in total clearance and volume of distribution of carbamazepine, resulting in no changes in carbamazepine half-life. Changes in the free carbamazepine concentration, however, influence the pharmacological effects and toxicity of the drugs. Drugs which undergo hepatic metabolism are modified by phase I reactions (oxidation, reduction, and hydroxylation) and/or phase II reactions (glucuronidation, acetylation, and sulphation) (Dawling & Crome 1989). Carbamazepine is oxidatively metabolized mainly by hepatic microsomal cytochrome P450 isoenzymes (P450 IIB or P450 IIC subfamily) to form carbamazepine-10, 11-epoxide (Liu & Delgado 1994). Phase I reactions are affected by the maturity compared with phase II reactions which are not (Greenblatt et al 1989). In addition, it has been reported that intrinsic clearance of carbamazepine shows a negative correlation with age (Liu & Delgado 1994).

Our previous study (Koyama et al 1997) showed that valproic acid displaced carbamazepine from the binding sites on albumin molecules. In our study, 38·5% of child and 48·5% of adult subjects were concomitantly administered valproic acid,

whereas elderly subjects received carbamazepine only. The child and adult groups were predicted to have elevated carbamazepine free fraction (%) compared with the elderly subjects, but this study showed significant elevation of carbamazepine free fraction (%) in the elderly group. This result suggested that the decrease in non-glycated albumin contributed greatly to the elevation of carbamazepine free fraction (%).

Thus, the marked increase in carbamazepine free fraction (%) in the elderly group was associated with decreased non-glycated albumin levels caused by decreased albumin levels and powerful glycation. This induces elevated carbamazepine concentrations in tissues expressing the receptor responsible for carbamazepine. In addition, elderly patients generally undergo multiple drug therapy, increasing the likelihood of drug interactions, which increasingly complicates carbamazepine kinetics in elderly patients. This can explain the increased incidence of adverse effects in elderly patients, and the increased risk of adverse effects necessitates reduction of the carbamazepine dose. To maximize the therapeutic benefits and to minimize the risk of adverse effects in elderly patients, the dosage should be adjusted by monitoring the free carbamazepine concentrations which directly reflect its pharmacological effects, rather than using the conventional total carbamazepine concentrations in the serum.

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