Covalent Binding of a Bucillamine Derivative with Albumin in Sera from Healthy Subjects and Patients with Various Diseases

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Purpose. To investigate the difference of pharmacokinetics of thiol-containing drugs in various disease states, we studied the covalent binding of SA3786, a bucillamine derivative, with proteins in patient serum compared with that in healthy serum.

Methods. Sera from healthy volunteers and patients of various diseases were supplied by the Japanese Red Cross Kumamoto Hospital. For the formation of conjugate experiments, SA3786 was added to a final concentration of 7×10^{-4}M. After 6h incubation at 37°C, HPLC analysis of 5 μl aliquots of each sample was performed using a column of N-methylpyridinium polymer (4VP-Me).

Results. The extent of HSA-SA3786 conjugate formation was found to be lower in the sera from healthy volunteers (control) than those from patients of various diseases. Especially high reactivity with SA3786 was observed in sera from rheumatic patients and hepatic patients. With the exception of the fraction of mercaptoalbumin (f_{HMA}), none of the parameters showed a good correlation with conjugate formation.

Conclusions. The parameter f_{HMA} must be considered to be one of the most important factors in formation of conjugates between plasma protein and thiol compounds. However, other factors may be involved in addition to f_{HMA} although the nature of these factors is not clear.

KEY WORDS: covalent binding; serum albumin; bucillamine; mercapto ratio.

INTRODUCTION

In the circulation, thiol-containing drugs bind covalently to plasma proteins, especially to serum albumin (1). Captopril (Cp), an angiotensin-converting enzyme (ACE) inhibitor, is a thiol-containing drug. Following intravenous administration, the half-life of unchanged Cp in blood was approximately 2h and about 80% of the dose was rapidly eliminated in urine within 6h in subjects with normal renal function (2,3). Thus, there may be no accumulation of unchanged Cp with multiple dosing because of its rapid elimination. However, after single and repeated dosing, the mean AUC values of total Cp rose from 15,475 to 24,505 μg/Lh (2). Further, this accumulation was more pronounced in disease states (4). The total Cp included Cp-protein (especially albumin) disulfide conjugation in addition to unchanged Cp, Cp-disulfide, Cp-Cys, or -GSH mix disulfide. The mean half-lives of penicillamine (PA) and PA-albumin conjugate were 0.59 and 40 h, respectively, following intravenous administration of PA to humans (5). These results suggested that covalent binding to plasma protein might be one of the factors of this accumulation.

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HSA is a heterogeneous mixture of mercaptoalbumin (HMA) and nonmercaptoalbumin (HNA) (6). HMA has only free SH in Cys^{34}, while HNA has no free SH groups, forming a mixed disulfide with Cys or oxidized (7,8). Only this Cys^{34} binds to thiol-containing drugs covalently. The fraction of HMA (mercapto ratio, f_{HMA}) decreases with aging and also in various disease states including chronic renal failure and rheumatism (9). This suggested that covalent binding of drugs with proteins in patients' sera may be different from that in healthy subjects'.

Thus, the present study was undertaken to investigate the covalent binding of thiol-containing drugs with proteins in patients sera compared with that in healthy sera. The differences in reactivity between diseased and normal sera will be discussed on the basis of serum biochemical data. In this study, the bucillamine (BA) derivative, N-(3,3-dimethyl-1-mercapto butyryl)-S-methyl-L-cysteine (SA3786), was used as a model thiol compound, because we can detect the HSA-SA3786 conjugate easily than any other thiol compond using 4VP-Me column.

MATERIALS AND METHODS

Sera from healthy volunteers and patients of various diseases were kindly supplied by the Japanese Red Cross Kumamoto Hospital (Kumamoto, Japan). The f_{HMA} was calculated by dividing the area under the peak corresponding to HMA by the total HSA area. For the formation of conjugate experiments, the serum samples were preincubated at 37°C, and N-(3,3-dimethyl-1-mercapto butyryl)-S-methyl-L-cysteine (SA3786) was added to a final SA3786 concentration of 7×10^{-4}M. After 6h incubation, HPLC analysis of 5 μl aliquots of each sample was performed using a column of N-methylpyridinium polymer (4VP-Me). The HPLC system was comprised of an LC-4A pump (Shimadzu, Tokyo, Japan) equipped with a gradient programmer and Shimadzu SPD-2AS UV monitor (10). The HSA fraction was eluted with a 30min linear gradient from 0 to 0.5M sodium chloride in 0.05M Tris-AcOH buffer (pH6.5) at a flow rate of 0.5ml/min at 25°C with detection at 280nm. In the absence of SA3786, the chromatograms of all sera were not changed after 6h incubation.

RESULTS AND DISCUSSION

The relative percentages of HSA-SA3786 conjugate formation in serum samples after 6h incubation are listed in Table I along with some clinical data. The extent of HSA-SA3786 conjugate formation was lower in the sera from healthy volunteers (control) than in patients with various diseases. Especially high reactivity with SA3786 was observed in sera from rheumatic patients (rheumatism) and from hepatic patients (hepatitis). In chronic renal failure patients' sera, the reactivity was decreased after hemodialysis. The relative percentages of HSA-SA3786 conjugate formation before and after hemodialysis (BHD and AHD) were 13.9 and 9.2%, respectively.

It is known that the f_{HMA} decreases with aging and in various disease states such as chronic failure, rheumatism etc. (9). Due to the low correlation coefficient between f_{HMA} and formation of conjugate ([HSA-SA3786(%)] = $29.59 - 0.315 \times [f_{HMA}(\%)]$; $r = 0.600$), we considered another factor which might be involved in the reaction. Thus, we examined the relationships between conjugate formation and other

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Table I. Clinical Data of Sera from Healthy Volunteers and Patients

Subject	Formation of complex	f_{HMA}	GOT	GPT	BUN	Creatinine	TG
	(%)	(%)	(IU/L)	(IU/L)	(mg/dL)	(mg/dL)	(mg/dL)
Healthy volunteers (n = 8)	11.7 ± 1.7	52.5 ± 6.2	18.1 ± 4.9	12.5 ± 3.0	14.9 ± 2.3	0.97 ± 0.16	80.0 ± 28.8
Before dialysis[a] (n = 8)	13.9 ± 2.3[b]	37.3 ± 7.8[c]	11.4 ± 6.2[b]	12.8 ± 6.5	70.4 ± 12.7[c]	12.00 ± 1.50[c]	77.3 ± 31.9
After dialysis[a] (n = 8)	9.3 ± 1.5[d]	52.4 ± 3.7[d]	15.5 ± 6.4	14.8 ± 7.7	22.8 ± 5.3[c,d]	4.78 ± 0.75[c,d]	54.9 ± 19.1[b]
Diabetes (n = 11)	15.1 ± 2.5[c]	50.6 ± 4.5	20.5 ± 8.9	18.5 ± 13.1	18.0 ± 3.7[b]	0.91 ± 0.22	150.2 ± 90.3[b]
Hepatitis (n = 6)	18.8 ± 4.5[c]	35.4 ± 11.3[c]	63.2 ± 35.3[b]	44.8 ± 28.9[b]	18.2 ± 4.0	1.10 ± 0.17	63.7 ± 24.1
Rheumatism (n = 11)	23.4 ± 4.7[c]	35.9 ± 10.5[c]	28.1 ± 14.2[b]	31.2 ± 46.2	18.8 ± 8.5	1.13 ± 0.59	114.7 ± 50.7

Note: All values are means ± SD.
[a] These serum were from renal chronic failure patient (CRF).
[b] $p < 0.05$.
[c] $p < 0.01$; for difference against healthy volunteeers.
[d] $p < 0.01$; for difference between before and after hemodialysis.

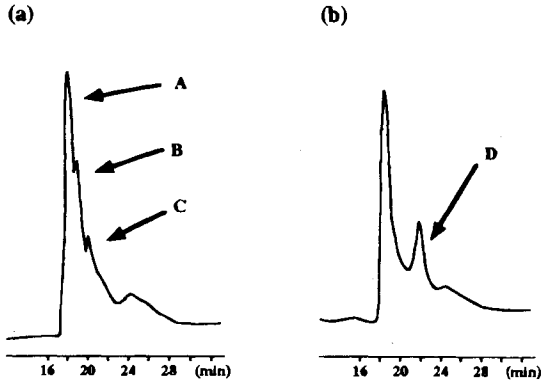

Fig. 1. Chromatograms of sera from healthy volunteers incubated with SA3786 for 0h (a) and 6h (b) A; mercaptoalbumin, B; non-mercaptoalbumin (-SS-Cys), C; non-mercaptoalbumin (oxidized form), D; HSA-SA3786 conjugate.

parameters. With the exception of f_{HMA}, none of the parameters showed a good correlation with conjugate formation (data not shown). Previously, we demonstrated that several factors such as pH, fatty acids, endogenous thiol, metal ions, and oxygen affected the formation of HSA-SA3786 conjugate in HSA solution (10). However, in this experiment, we observed no good correlation between these parameters and conjugate formation. From these results, f_{HMA} must be considered to be one of the most important factors in the formation of conjugates between plasma protein and thiol compounds. However, covalent binding could not be explained fully by the f_{HMA} values. This suggests that other factors may be involved in addition to f_{HMA}, although the nature of these factors was not clear.

HNA heterogeneity might be such an additional factor. There was marked heterogeneity of HNA in patients' sera. HNA was a mixture of oxidized HSA and HSA-thiol (Cys or glutathione) conjugate. In patients with rheumatism, hepatitis, and renal failure, the increase in HNA might be due to the increase in the levels of HSA-thiol conjugate rather than oxidized HSA. In contrast, in diabetes and aging, the increases in conjugate formation can be explained by oxidized HSA (11). The effect of heterogeneity of HNA might be less than that of f_{HMA}, but these parameters would still have important effects.

To demonstrate the complexity of this reaction and to clarify the importance of f_{HMA} for conjugate formation, only the relationships between formation of conjugate and biochemical parameters in BHD and AHD were analyzed because the mechanism of covalent binding in a specific disease state might be easily elucidated rather than by examining various diseases. Several parameters such as free fatty acids (0.22 and 1.05mEq/L,BHD and AHD, respectively), BUN (70.4, 22.8mg/dL, respectively), creatinine (12.0, 4.8mg/dL, respectively), and f_{HMA} (37.3, 52.4%, respectively) were changed significantly by hemodialysis. In fact, a better correlation for f_{HMA} was observed ($[\text{HSA-SA3786}(\%)] = 22.38 - 0.241 \times [f_{HMA}(\%)]$; $r = 0.769$) (Fig. 2). This clearly indicates that many factors affect this reaction. These results support our hypothesis that f_{HMA} is an important factor for conjugate formation in serum.

In general, thiol-containing drugs bind to tissue protein through SH/SS interactions, and these drugs remain at relative high levels in tissues such as skin, gastric mucosa, vascular wall, and cartilage (12). Clinically, thiol compounds have various adverse effects such as skin rash, gastricism, protein urea, agranulocytosis, and nephrotic syndrome (1,13); moreover, dyshepatia is also observed rarely (14). In several reports, it was suggested that these adverse effects might be strongly related to conjugation with tissue protein (15). Therefore, the findings obtained here may be useful for future clinical, pharmacological, and pharmacokinetic studies of thiol-containing drugs.

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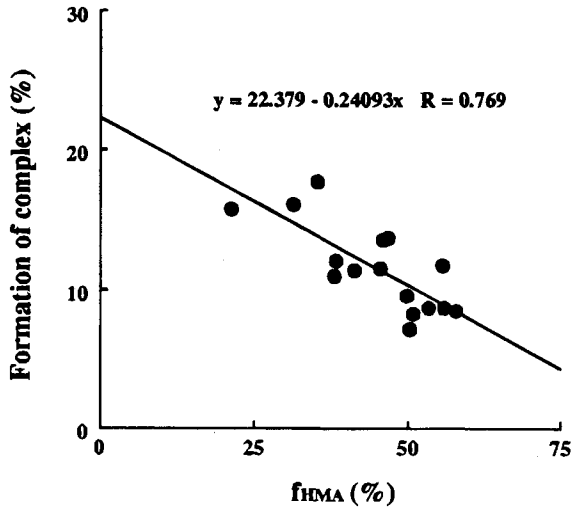

Fig. 2. Correlation between f_{HMA} and the formation of HSA-SA3786 conjugate in CRF patients.

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REFERENCES

1. Park, B., Grabowski, P., Yeumg, J., and Brekenridge, A. *Biochem Pharmacol* **31**:1755–60 (1982).
2. Duchin, K., McKinstry, D., Cohen, A., and Migdalof, B. *Clin Pharmacokinet* **14**:241–59 (1988).
3. Drummer, O., Workman, B., Miach, P., Jarrott, B., and Louis, W. *Eur J Clin Pharmacol* **32**:267–71 (1987).
4. Cody, R., Schaer, G., Covit, A., Pondolfino, K., and Williams, G. *Clin Pharmacol Ther* **32**:721–6 (1982).
5. Joyce, D., and Day, R. *Br J Clin Pharmac* **30**:511–7 (1990).
6. Petersen, H., and Foster, J. *J Biol Chem* **240**:2503–7 (1965).
7. King, T. *J Biol Chem* **236**:PC5 (1961).
8. Wallevik, K. *Biochim Biophys Acta* **420**:42–56 (1976).
9. Sogami, M., Era, S., Nagaoka, S., Kuwata, K., Kida, K., and Shigemi, J. *J Chromatogr* **332**:19–27 (1985).
10. Narazaki, R., Hamada, M., Harada, K., and Otagiri, M. *Pharm Res* **13**:1317–21 (1996).
11. Suzuki, E., Yasuda, K., Takeda, N., Sakata, S., Era, S., Kuwata, K., Sogami, M., and Miura, K. *Diabetes Res Clin Practice* **18**:153–8 (1992).
12. Lan, S., Weinstein, S., and Migdalof, B. *Drug Met Disp* **10**:306–11 (1982).
13. Yeung, J., Breckenridge, A., and Park, B. *Biochem Pharmacol* **32**:2467–72 (1983).
14. Park, B., Pirmohamed, M., and Kitteringham, N. *Br J Clin Pharmac* **34**:377–395 (1992).
15. Park, B., and Kitteringham, N. *Drug Metab Rev* **22**:87–144 (1990).