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Influence of Albumin Glycation on the Protein Binding of Drugs

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ABSTRACT

Glycation occurs by covalent binding between the carbonyl group of monosaccharides and the epsilon amino group of amino acid. It can alter the physiological function of proteins and causes the development of diabetic complications. In this study, the influence of glycation on protein binding of warfarin and dansylsarcosine was studied by equilibrium dialysis which was performed for 3 hours at 37℃ in the water bath. The high glycated albumin which contained 50±16% of glycated albumin bound less than natural albumin which contained 8.5±5.28% of glycated albumin, if drugs concentration were more than the albumin concentration. But only warfarin binding showed a significant difference of 6% ($P<0.05$) when the molar concentration ratio of warfarin per albumin was 3. In consideration of low therapeutic concentrations, low glycated albumin concentrations in the body, and rapid elimination of excessive free drugs, these small increaes of free warfarin concentrations by glycation of albumin are not considered as risk factors for drug intoxication for diabetics, if renal functions are intact.

Key Words: Glycation, Protein binding, Albumin, Equilibrium dialysis

INTRODUCTION

Monocarbohydrates are incoperated in serum albumin and other proteins by covalent binding between the carbonyl group of monosaccharides and epsilon amino group of amino acid, especially lysine and valine. This reaction is called glycation or nonenzymatic glycosylation. Its reaction velocity depends on the kinds of monosaccharide and its concentration, reaction temperature, and acidity, etc. (Cohen, 1985; Furth, 1983).

The glycation of proteins results in a variation of their physiological function by changing the molecular structure (Flecha *et al.*, 1990; McMillan *et al.*, 1981; Baba *et al.*, 1981; Bilan *et al.*, 1990; McDonald *et al.*, 1979; Williams *et al.*, 1982; Shakalai *et al.*, 1984; Stevens *et al.*, 1978; Sensi *et al.*, 1989; Rosenberg *et al.*, 1979; Tarsio *et al.*, 1985; Cortiz *et al.*, 1991; Duell *et al.*, 1990). These alterations are known as the causes of the development of diabetic complications (Kennedy *et al.*, 1984).

As an important carrier protein of drugs and metabolites, albumin has specific drug binding sites for azapropazon-and warfarin, indol-and benzodiazepin and digitoxin. Some drugs such as aspirin, tolbutamide bind to albumin relative unspecifically (Müller, 1982).

There were some reports that glycation of serum protein inhibited protein binding of drugs. But most of these studies were performed with diabetic serum which differed from healthy serum in its composition, such as glycated protein, free fatty acid, and other endogenous metabolites etc. which can influence the protein binding of durgs.

(Bae, 1994, Colombo *et al.*, 1982; Gatti *et al.*, 1987, McNamara *et al.*, 1988; Storck *et al.*, 1991, Wörner *et al.*, 1992). To examine the effect of glycation on protein binding of drugs, it was required to use very different glycated protein which could be produced from pure albumin in vitro. So, free warfarin and dansylsarcosine(DS) concentration, as representative drugs, for main albumin binding sites were compared after incubation of bovine serum albumin (BSA) with glucose by using equilibrium dialysis (Menke *et al.*, 1989).

MATERIALS AND METHODS

Preparation of phosphate buffer saline (PBS)

0.067 M PBS was produced from mixing of 0.067 M disodium hydrogenphosphate and 0.067 M mono-sodium dihydrogenphosphate resulting in pH 7.4, 6 g of sodium chloride and 0.975 g of sodium azide were dissolved in a volume of 1 liter of PBS.

Preparation of high glycated and natural albumin solution

For the production of high glycated albumin solution, 10 g of fatty acid free bovine serum albumin (BSA) (Sigma A-6003) and 10 g glucose were dissolved in PBS to become the final volume of 200 ml. After filtration of this solution by 0.2 μm sterile syringe filter (Nalgene, USA), 100 ml solution was incubated for 15 days at 37°C water bath with slight shaking (Julabo, Germany) and then dialysed by dialysis tube (MWCO 12,000~14,000, Spectrapore) against 2000 ml PBS for 3 times within 24 hours to eliminate the free glucose. For the production of natural albumin solution, another 100 ml albumin solution was dialysed without the incubation process. The 3rd dialysis of each albumin solutions were done in the same beaker to equilibrate the osmotic pressure of each solutions. These albumin solutions were diluted with PBS to 160 uM. The concentration of albumin was determinded by 280 nm from spectrophotometer (Kontron 806, Swiss).

Determination of glycated albumin concentration

Glycated albumin concentration was determinded for seven times by affinity chromatography column which were filled with m-aminophenylboronic acid agarose (Sigma A-8530). The analytical process was performed by Pierce Glyco-Gel test.

Eqilibrium dialysis

The degree of drug-protein binding was measured by equilibrium dialysis (Scholtan, 1978) with a Spectrum apparatus (Model 08-681-2B). The dialysis cell was seperated by dialysis menbrane disc of MWCO 5000 (Spectropore, USA). 1 ml of albumin solutions were injected in one side and 1 ml of variable concentrated drugs were injected in the other side. Thereafter, dialysis cells were rotated with 12 times/minute in 37° C water bath for 3 hours. An aliquot was taken from the drug side for the determination of the free drug concentration. Equilibrium dialysis was performed six times for each samples.

Determination of drug concentration

Drugs and albumin concentration were measured by spectrometrically determinded equilibration curves (Kontron 806, Swiss). The peak waves length were decided by scanning process.

Statistic evaluation

Statistic significance of bound drugs concentration per albumin between high glycated and natural albumin were evaluated by t-test.

RESULTS

Determination of glycated albumin concentration

High glycated albumin solution containd 50±16 % (n=7) glycated protein by incubation with 10 g glucose and 10 g albumin in 200 ml PBS for 15 days at 37°C water bath. Natural albumin solution contained 8.5±5.28% (n=7) of glycated protein which was suggested to be produced in the body (Mean±S.D.).

Equilibration curve for warfarin, dansylsarcosine and albumin

Fig. 1, 2 and 3 show the equilibration curve of warfarin, dansylsarcosine and albumin. For the

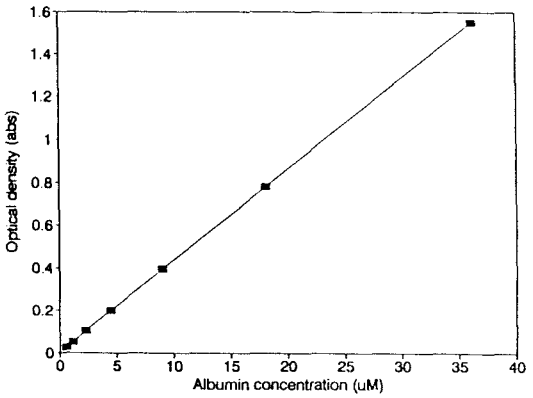

Fig. 1. Equilibration curve of bovine serum albumin. The optical density were determinded at 280 nm and albumin was dissolved in 0.067 M PBS.

determination of these concentration, 280 nm for albumin, 310 nm for warfarin and 328 nm for dansylsarcosine were used. The correlation degree for all equilbration curves were more than 0.99.

Binding of warfarin to albumins

Table 1 and Fig. 4 show the binding of warfarin to high glycated and natural albumin solutions. Molar concentration ratios of bound warfarin per albumin were increased by increasing of total warfarin concentration. But molar concentration ratios of warfarin per natural albumin bound were a little hgiher than per high glycated albumin bound if molar concentration ratio of total warfarin per albumin were 3 and 1.5. But only warfarm-binding was decreased 6% significantly

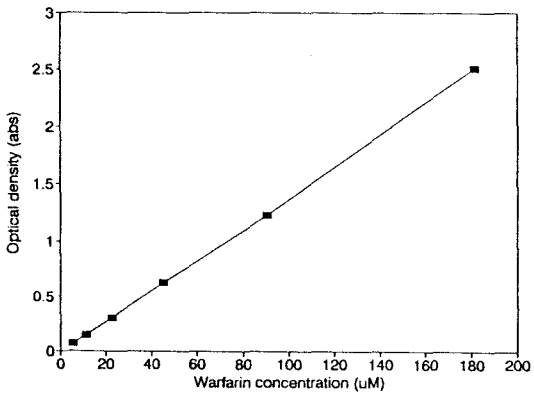

Fig. 2. Equilibration curve of warfarin. The optical density were determinded at 310 nm and warfarin was dissolved in 0.067 M PBS.

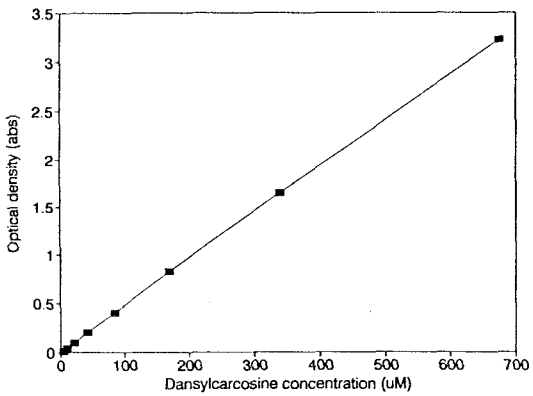

Fig. 3. Equilibration curve of dansylsarcosine. The optical density were determined at 328 nm and dansylsarcosine was dissolved in 0.067 M PBS.

Table 1. The results of the binding test of warfarin and dansylsarcosine to glycated albumin (50±15% glycated albumin contained) and natural albumin (8.5±5.28% glycated albumin contained) by equilibrium dialysis. Each determination was performed 6 times (Mean±S.D.)

Warfarin				Dansylsarcosine			
Ctotal-drug / Calbumin	Cbound-drug/Calbumin		P-value	Ctotal-drug / Calbumin	Cbound-drug/Calbumin		P-value
	Glycated albumin	Natural albumin			Glycated albumin	Natural albumin	
3	2.015±0.044	2.135±0.048	0.0012	8.8	4.100±0.411	4.450±0.145	0.0952
1.5	1.183±0.067	1.242±0.029	0.0905	4.4	2.470±0.201	2.582±0.082	0.2516
0.75	0.668±0.038	0.653±0.041	0.5257	2.2	1.585±0.059	1.551±0.039	0.2812
0.375	0.338±0.028	0.338±0.020	1.000	1.1	0.918±0.054	0.890±0.021	0.2718
0.1875	0.172±0.020	0.162±0.010	0.305	0.55	0.461±0.018	0.465±0.019	0.7618

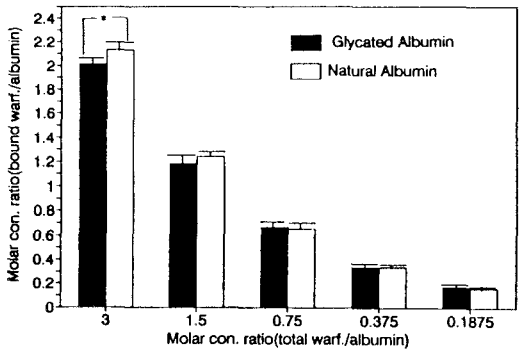

Fig. 4. Molar concentration of warfarin per albumin bound between 3 and 0.1875 of molar concentration ratio of total warfarin per albumin (Mean±S.D., n=6) *p<0.05.

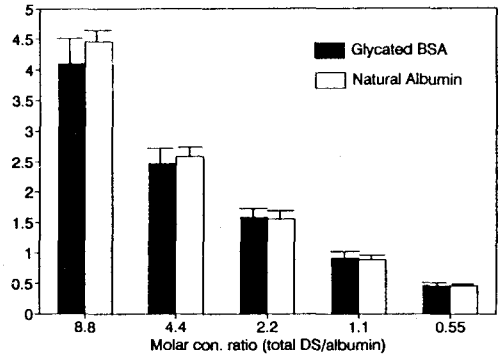

Fig. 5. Molar concentration of dansylsarcosine per albumin bound between 8.8 and 0.55 of molar concentration ratio of total warfarin per albumin (Mean±S.D., n=6).

(P<0.05), if the molar concentratcon ratio of total warfarin/albumm was 3.

Binding of dansylsarcosine to albumins

Table 1 and Fig. 5 show the binding of dansylsarcosine to high glycated and natural albumin solution. Molar concentration ratio of bound dansylsarcosine per albumin was increased by increasing of the total dansylsarcosine concentration. But molar concentration ratio of dansylsarcosine per natural albumin bound were a little more than per high glycated albumin bound if molar concentration ratio of total dansylsarcosine per albumin were 8.8 and 4.4. But these differences were not significant.

From these results natural albumin bound more drugs than glycated albumin when molar concentration ratios of total drugs per albumin were over 1. But only warfarin binding showed a significant difference when its molar concentration ratios of total warfarin per albumin was 3.

DISCUSSION

Because free drugs are only pharmacological active, increasing of free drug concentration can cause enhanced drug toxicity (Aarons *et al.*, 1979). Significantly lower association velocity and the affinity constants of dansylsarcosine (Worner *et al.*, 1992), reduced protein binding of prazosin (Trovik *et al.*, 1992) and sulfioxazole (Ruiz-Cabello *et al.*, 1984) in diabetic serum indicate the alteration of protein binding of drugs. Diabetic serum contains high concentration of free fatty acid, glycated plasma protein and the other endogenous substance which can influence the protein binding of drugs. To examine the effect of glycation on protein binding of drugs is necessary to produce high glycated protein from pure albumin. Therefore, fatty acid free bovine serum albumin was incubated for 15 days in this study. Though the glycated albumin concentration in blood varies between specise, observer, assay methods and individual condition, it was usually less than 10% for health person and 10~30% diabetic person (Dolfer *et al.*, 1880; Gutthrow *et al.*, 1979). The great difference of glycated albumin concentration with 8.5%±5.28% for natural albumin solution and 50±18% for high glycated albumin solution can help to clarify the effect of glycation on the protein binding of drugs.

Seperation of free drugs were done by equilibrium dialysis which is considered as a standard method for protein binding of ligands. To faciliate distribution of free drugs, 0.6% sodium chloride was added in buffer to inhibit the Gibbs-Donnan effect. Dansylsarcosine used a model ligand for the benzodiazepine binding site. Because these drugs reached within 1.5 hours to equilibrated concentration in pretest, equilibrium dialysis for 3

hours is thought to be enough time to reach to equilibrate concentration in two side of dialysis cell.

Even though the high glycated albumin bound with warfarin and dansylsarcosine less than natural albumin if the molar concentration ratio of total drug per albumin was above 1 (Fig. 4, 5), only warfarin showed significant difference when molar concentration ratio was 3. This result indicates that glycation inhibit the protein binding of drugs, especially for warfarin binding site bound drugs. The linear correlation ($r=0.49$, $P=0.014$, $y=0.3x+3.7$) was found between the extent of glycated albumin and the free fraction of phenytoin in serum (Kerns *et al.*, 1988). Free drug concentration of sulfisoxazole (Ruiz-Cabello *et al.*, 1984) and gliclazide (Igaki *et al.*, 1992) were increased by glycation of plasma protein. Because these drug bind to the warfarin binding site of albumin (Müller, 1982), these results are thought to be consistant with this study. Increasd free drugs concentration of diazepam and valproic aicd in diabetic serum were considered to result from more free fatty acid than glycated protein (Ruiz-Cabello *et al.*, 1984, Gatti *et al.*, 1987), so dansylsarcosine as model ligand for diazepam is thought to be not significantly bound in this study.

In consideration of therapeutic plasma concentration which is usually less than equilmolar concentration with blood albumin, less glycated albumin concentration of diabetic subjects than high glycated albumin of this study and rapid renal clearance of increased free drug, the glycation of albumin is not considered as risk factor for drug intoxication by increased free drug concentration in the body.

Therefore, the glycation of albumin is not considered as risk factor for drug intoxication in the body, if renal function was intact.

REFERENCES

Aarons LJ, Schary WL and Rowland M: *An in vitro study of drug displacement interactions: Warfarin-salicylate and warfarin-phenylbutazone. J Pharm Pharmacol 31: 322-330, 1979*

Bae JW: *Influences of free fatty acid on the blbumin binding of warfarin and dansylsarcosine. Korean J Pharmacol 30(2): 225-60, 1994*

Baba Y, Motoaki K and Kamada T: *Erythrocyte membrane microviscosity in diabetes. Horn Metab Res Suppl II: 97-102, 1981*

Bilan PJ and Klip A: *Glycation of the human erythrocyte glucose transporter in vitro and its functional consequences. Biochem 268(2): 66-7, 1990*

Cohen MP: *Diabetes and protein glycation. Springer verlag: 5-14, 1985*

Colombo R and Pinelli A: *Effect of in vivo increase of fee fatty acids on human plasma protein binding of furosemide. Pharmacology 25(2): 73-81, 1982*

Cortiz VM and Gagliardino JJ: *Protein glycation: Its role in the changes induced by diabetes in the properties of the serum insulin-like growth factor-I binding proteins. J Endocrinol 131(1): 33-38, 1991*

Dolhofer D and Wieland OH: *Increased glycosylation of serum albumin in diabetes mellitus. Diabetes 29(June): 417-422, 1980*

Duell PB, Oram JF and Bierhan EL: *nonenzymatic glycosylation of HDL resulting in inhibition of high-affinity binding to cultured human fibroblasts. Diabetes 39: 1257-1264, 1990*

Flecha FLG, Bermudez MC, Cedola NV, Gagliardind JJ and Rossi JPFC: *Decreased Ca^{++}-ATPase activity after glycosylation of erythrocyte membranes in vivo and vitro. Dibetes 39: 707-711, 1990*

Furth AJ: *Methods for assaying nonenzymatic glycosylation. Analytical Biochem 175: 347-360, 1988*

Gatti G, Crema F, Attardo-Parrinelo G, Fratino P, Aguzzi Fk and Perucca E: *Serum protein binding of phenytoin and valproic acid in insulin-dependent diabetes mellitus. Ther-Drug-Monit 9(40: 389-91, 1987*

Gutthrow CE, Morris MA, Day JF, Thorp SR and Baynes JW: *Enhanced nonenzymatic glycosylation of human serum albumin in diabetes mellitus. Proc Natl Acad Igaki A, Kobayahi K, Kimura M, Sakoguchi T, Matsuoka A: Influence of blood proteins on biochemical analysis. Chem-Pharm-Bull 40(1): 255-7, 1992*

Kennedy L and Baynea JW: *Non-enzymatic glycosylation and the chronic complication of diabetes: an overview. Diabetologia 26: 93-98, 1984*

Kerns GL, Kemp SF, Turley CP and Nelson DL: *Protein binding of phenytoin and lidocaine in pediatric patients with type 1 diabetes mellitus. Dev-Pharmacol-Ther 11 (1): 14-23, 1988*

McDonald MJ, Bleichman M, Bunn HF and Noble RW: *Functional properties of glycosylated minor components of human adult hemoglobin. J Biol Chem 254: 702-707, 1979*

McMillan DE, Utterback NG, Sparks LL and Bramwell PC: *Imparied doublet formation in diabetes. Diabe-*

tologia 21: 575-578, 1981

McNamara PJ, Blouin RA and Brazzel RK: *The protein binding of phenytoin, propranolol, diazepam, and AL01576 in human and rat diabetic serum. Pharm-Res 5(5): 262-5, 1988*

Menke G, Worner W, Kratzer W and Rietbrock N: *Kinetics of drug binding to human serum albumin: Allosteric and competitive inhibition at the benzodiazepine binding site by free fatty acids of various chain lengths. Naunyn-Schmiedeberg's Arch Pharmacol 339: 42-47, 1989*

Muller WE: *Die Plasmaproteinbindung von Pharmaka. Med Mo Pharm 5. Jahrgang Heft 10: 302-310, 1982*

Rosenberg H, Modrak JB, Hassing JM, Al-Turk WA and Stohs SJ: *Glycosylated collegen. Biochem Biophys Res Commun 91: 498-501, 1979*

Ruiz-Cabello F and Erill S: *Abnormal serum protein binding of acidic drugs in diabetes mellitus. Clin Phramacol Ther 35(5): 691-695, 1984*

Scholtan W: *Bestimmungsmethoden und gesetzmaessigkeiten der serumproteinbindung von Arzneimitteln. Arzneim. -Forsch./Drug Res 28(11): 1037-1047, 1978*

Sensi M, Tanzi P, Bruno MR, Pozzilli P, Mancuso M, Gambardella G and Di Mario U: *Nonenzymic glycation of isolated human glomerular basement changes its physicochemical characteristics and binding properties. Nephron 222-226, 1989*

Shaklai N, Garlick RL and Bunn HF: *Nonenzymatic glycosylation of human serum albumin alters its conformation and function. J Biol Chem 259(6): 3812-3817, 1984*

Stevens VJ, Rouzer CA, Monnier VM and Cerami A: *Diabetic cataract formation: Role of glycosylation of lens crystallins. Proc Natl Acad Sci USA 75: 2918-2922, 1978*

Storck J and Kirsten R: *Binding of urapidil to heman serum albumin: dependency on free fatty acid concentration. Int-J-Clin Pharmacol-Ther-Toxicol 29(5): 204-8, 1991*

Tarsio JF, Wigness B, Rhode TD, Rupp WM, Buchwald H and Furcht LT: *Nonenzymatic glycation of fibronectin and alterations in the molecular association of cell matrix and basement membrane components in diabetes mellitus. Diabetes 34(5): 477-84, 1985*

Trovik TS, Jaeger R, Jorde R, Ingebrestsen O and Sager G: *Plasma protein binding of catecholamines, prazosin and propranolol in diabetes mellitus. Eur-J-Clin-Pharmacol 43(3): 265-8, 1992*

Worner W, Preissner A and Rietbrock N: *Drug-protein binding kinetics in patients with type I diabetes. Eur-J-Clin-Pharmacol 43(1): 97-100, 1992*

=국문초록=

알부민 Gylcation이 약물의 단백질결합에 미치는 영향

건국대학교 의과대학 약리학교실

배 진 우

Glycation이란 단당류의 카보릴기와 아미노산의 입실론 아미노기가 공유결합에 의하여 형성되는 반응으로 이는 단백질의 생리적 기능을 변화시키며 아울러 당뇨합병증을 유발한다. 본 연구에서는 warfarin과 dansylsarcosine의 단백질결합에 미치는 glycation의 영향을 평형투석법을 이용하여 연구하였으며 평형투석은 섭씨 37도의 진탕수조에서 3시간 동안 실시하였다. 약물의 농도가 알부민의 농도보다 높을 경우, 50±16%가 glycation된 알부민은 8.5±5.28% glycation 알부민을 함유한 정상알부민에 비해 약물과의 결합도가 낮았으나 warfarin의 농도가 알부민의 3배가 될 경우에만 유의성이 인정되었고($P<0.05$) 6%의 차이를 보였다. 본 실험에 나타난 glycation에 의한 유리약물의 미미한 상승효과는 glycated albumin 농도가 낮은 생체내의 여건과, 실제로 사용되는 약물의 적정 치료농도가 낮고 또한 과도한 유리약물은 신장을 통하여 신속히 배설되는 이유로 당뇨환자의 신기능 손상이 없는한 glycation에 의한 유리약물의 상승은 약물중독의 위험요소로 작용되지 않으리라 생각된다.