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POSTTRANSLATIONAL CHANGES OF ALBUMIN AS A CAUSE OF ALTERED DRUG-PLASMA PROTEIN BINDING

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Experimentally induced posttranslational changes in serum albumin have repeatedly been used to study binding sites on this protein, but less attention has been paid to qualitative changes of albumin in disease states. These modifications could be due to reactive chemicals formed in the disease process or to pharmacological agents used in its management.

UREMIA

There is now a body of information suggesting that endogenous displacers play a major role in the plasma protein binding defect in uremia,[21] but there is also evidence implicating a qualitative change of the albumin molecule in this condition.[25] In view of the spontaneous formation of cyanate from urea in solution,[9] and the reactivity of this chemical with amino groups in proteins,[29] some years ago we evaluated the in vitro effects of potassium cyanate on drug-protein binding.[10] Carbamylation of normal plasma was readily achieved in vitro, and it could be shown that the binding of an acidic drug—salicylate—was decreased in carbamylated plasma, while that of a basic drug—quinidine—was unaffected.[11]

It had been reported that the amino acid composition of uremic serum albumin differs from that of normal serum, and that the decreased sulfadiazine binding exhibited by uremic serum could be characterized by a decrease in the affinity constant without modification in the number of binding sites.[4] Interestingly, this was also the case in the binding of this sulfonamide to carbamylated plasma (Fig. 17-1).

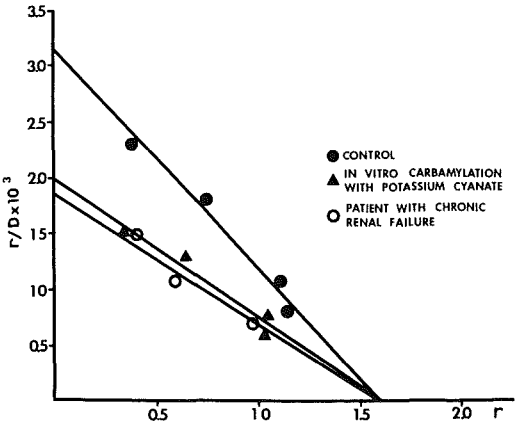

FIGURE 17-1
Representative Scatchard plots for sulfadiazine binding to plasma proteins in a pool of normal plasma before and after carbamylation with 40 mM potassium cyanate and in the plasma from a patient with uremia.
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A role for carbamylation of plasma proteins in the drug binding defect of uremia may seem to contrast with several studies reporting the lack of effect of urea, added in vitro, on the binding of dyes or drugs to plasma proteins,[6,27] but in these studies the incubation time was short, and it is well known that production of cyanate from urea proceeds slowly.[15] In an experiment in which plasma samples from five normal subjects were incubated with urea at 37°C for periods up to three weeks, carbamylation of plasma proteins was not detected before about one week and much higher degrees were observed after two and three weeks of incubation.[11] In another study, recently conducted, we measured the carbamylation of serum proteins in rabbits after renal failure induced by the intravenous injection of uranyl nitrate. In this case, the modification of serum albumin is minimal three days after the injection and, again, about a week is needed to observe degrees of protein carbamylation similar to those detected in uremia (Fig. 17-2). In this context, studies of experimentally-induced acute renal failure seem hardly adequate to evaluate the role of carbamylation in the binding defect of chronic uremia.

Although the binding of acidic drugs to their two main binding sites on albumin seems to be impaired in uremia,[25] experimental carbamylation of normal plasma decreases the binding of site I[1,5]

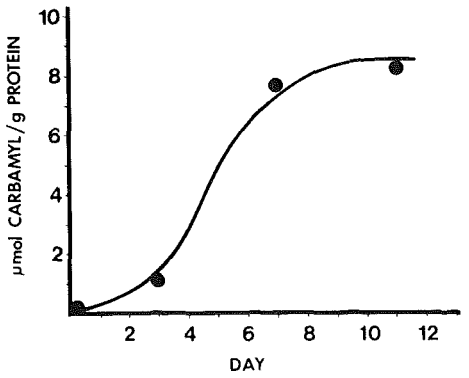

FIGURE 17-2
Carbamylation of plasma proteins in rabbits after experimental renal failure induced by the injection of 2 mg/kg uranyl nitrate.

but not of site II drugs[5] (Figs. 17-3 and 17-4). Using sulfisoxazole and diazepam as model drugs, we showed that charcoal treatment of uremic plasma fully restored the defective binding of the latter, but did not achieve full normalization of sulfisoxazole binding and, curiously enough, the binding defect after charcoal treatment could be accounted for by albumin carbamylation[5] (Table 17.1).

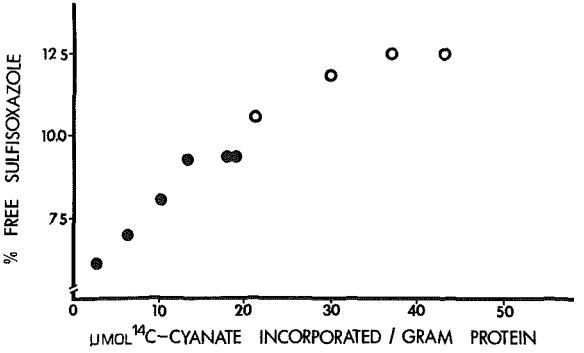

FIGURE 17-3
Changes in the free fraction of sulfisoxazole in plasma induced by in vitro carbamylation of plasma proteins with 40 mmol/L and 100 mmol/L potassium cyanate. ● = 40 mmol cyanate; o = 100 mmol cyanate.
Source: Calvo, Carlos, and Erill 1982. Reprinted with permission of Karger AG.

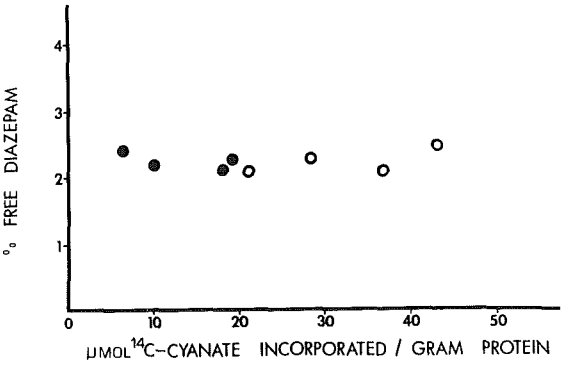

FIGURE 17-4
Free fraction of diazepam in plasma after in vitro carbamylation of plasma proteins with 40 mmol/L and 100 mmol/L potassium cyanate. ● = 40 mmol cyanate; o = 100 mmol cyanate.
Source: Calvo, Carlos, and Erill 1982. Reprinted with permission of Karger AG.

TABLE 17.1
Effect of Charcoal Treatment on the Binding of Sulfisoxazole (100 mg/L) and Diazepam (3 mg/L) to Normal Plasma, Carbamylated Normal Plasma, and Plasma from Uremic Patients (free fraction ± SEM)

	Sulfisoxazole (n = 6)		Diazepam (n = 6)	
	Before Charcoal Treatment (percent)	After Charcoal Treatment (percent)	Before Charcoal Treatment (percent)	After Charcoal Treatment (percent)
Normal	5.4 ± 0.6	5.9 ± 1.1	2.8 ± 0.2	2.6 ± 0.2
Carbamylated	9.7 ± 0.7	9.6 ± 0.8	2.6 ± 0.1	2.7 ± 0.1
Uremic	15.6± 3.0	9.6 ± 0.9	4.5 ± 0.6	2.2 ± 0.2

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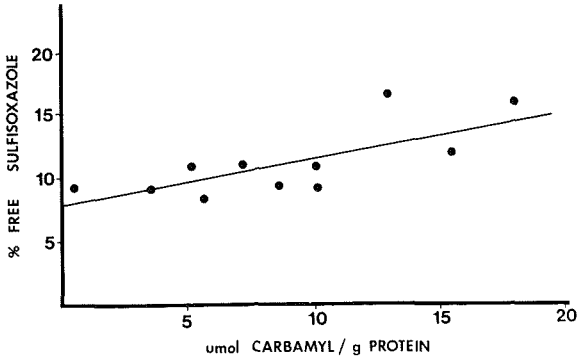

FIGURE 17-5
Graph of percentage sulfisoxazole in plasma/degree of carbamylation of plasma proteins in patients with uremia.

As it had been observed with salicylate, in a previous study,[11] there was a correlation between percentage free sulfisoxazole in plasma and degree of carbamylation of plasma protein, in a series of patients with renal failure (Fig. 17-5). Also in this case, abnormally high values of percentage free drug in the absence of protein carbamylation (y intersect) suggest that other factors, most likely the presence of endogenous displacers, also play a role.

The evidence supporting the existence of structural changes on albumin in uremic patients includes observation of abnormal behavior on isoelectric focusing,[27] changes in amino acid composition[4] and identification of substantial amounts of homocitrulline among its amino acids.[2] Homocitrulline is formed through carbamylation of lysine residues and these are believed to participate in drug site I on albumin.[14] Whatever its quantitative contribution, carbamylation of albumin in chronic uremia seems to offer an interesting example of the possible role of posttranslational modifications of proteins in disease-induced changes in the plasma protein binding of drugs.

DIABETES MELLITUS

Nonenzymatic glycosylation of proteins is common in diabetes mellitus,[19] and has been suggested to contribute to the long-term complications of the disease.[19,30,34] Serum albumin is readily glycosylated[8] and measurement of glycosylated albumin, or of glycosylated serum protein has been advocated for the estimation of diabetic control.[18] Lysine residues on albumin are involved in the

TABLE 17.2
Glycosylation of Plasma Proteins and Changes in Protein Binding of Sulfisoxazole (100 μg/ml) and Diazepam (3 μg/ml) in Normal Serum Samples Incubated at 37°C with 20 mM Glucose (N = 8)

	Day 0		Day 4		Day 8		Day 12	
	Control	Glucose	Control	Glucose	Control	Glucose	Control	Glucose
Glucose incorporated (mg/gm protein)	-	-	-	2.6 ± 0.1	-	3.3 ± 0.1	-	4.3 ± 0.2
Percent free sulfisoxazole	7.8 ± 0.6	7.9 ± 0.7	12.9 ± 1.7	14.3 ± 1.8*	18.2 ± 1.0	21.0 ± 1.5*	25.5 ± 2.0	30.3 ± 2.5†
Percent free diazepam	2.5 ± 0.2	2.3 ± 0.2	2.9 ± 0.1	3.3 ± 0.4	3.6 ± 0.2	4.6 ± 0.4	4.7 ± 0.4	4.9 ± 0.5

*$P < 0.01$
†$P < 0.001$

Source:
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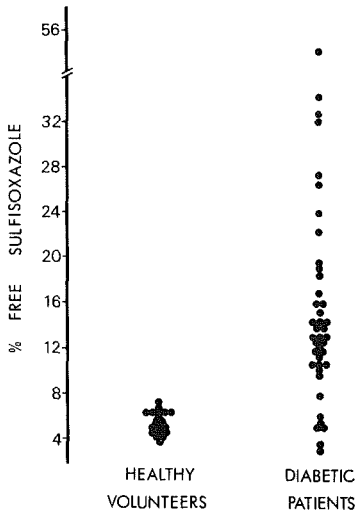

FIGURE 17-6
Percentage of free sulfisoxazole in serum of healthy subjects and of patients with diabetes mellitus.
Source: Ruiz-Cabello and Erill 1984. Reprinted with permission of The C. V. Mosby Co.

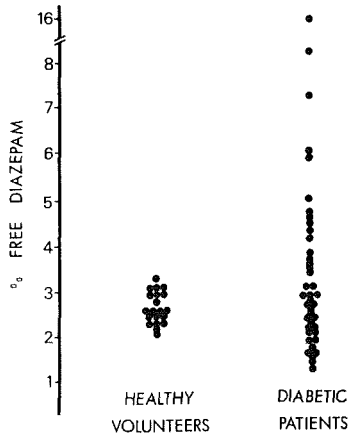

FIGURE 17-7
Percentage of free diazepam in serum of healthy subjects and of patients with diabetes mellitus.
Source: Ruiz-Cabello and Erill 1986. Reprinted with permission of The C. V. Mosby Co.

glycosylation and, therefore, it seemed of interest to investigate whether the binding defect produced by cyanate is reproduced in glycosylated plasma.

From data in Table 17.2, it seems evident that in vitro glycosylation of plasma proteins is associated with an increase in the free fraction of sulfisoxazole over that of control samples without added glucose, while the protein binding of diazepam is not affected.[26] When the plasma protein binding of these two model drugs was evaluated in patients with diabetes mellitus, an increase in the free fraction of sulfisoxazole (Fig. 17-6) and, to a lesser extent, of diazepam (Fig. 17-7) was detected. In the case of sulfisoxazole, there was a clear correlation (Fig. 17-8) between percentage free drug in serum and glycosylation of serum protein. Free fraction of diazepam did not seem related to the amount of glycosylated protein, and most of the samples of serum with high values of diazepam free fraction also had abnormally high levels of free fatty acids. Removal of free fatty acids from serum by charcoal treatment consistently decreased the percentage of free diazepam while not modifying, or even increasing, the percentage of free sulfisoxazole in serum.[19]

In view of the similarities in the binding defect induced by glycosylation and carbamylation of plasma proteins, a competition experiment was performed. Samples of normal serum were carbamylated by incubation with 10 mM potassium cyanate for varying periods of time and afterwards dialyzed at 4°C against (pH 7.4) saline. Dialyzed samples were then incubated under sterile conditions with

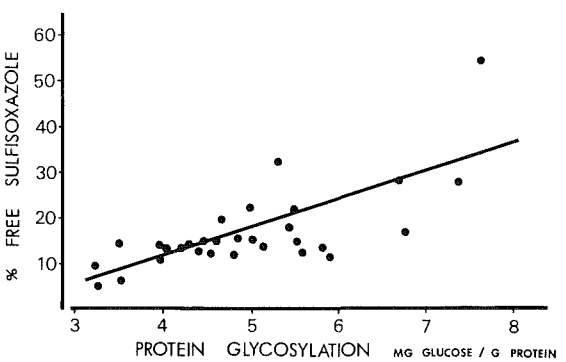

FIGURE 17-8
Percentage of free sulfisoxazole in serum plotted against the degree of glycosylation of serum protein in patients with diabetes mellitus not treated with oral antidiabetic drugs.
Source: Ruiz-Cabello and Erill 1984. Reprinted with permission of The C. V. Mosby Co.

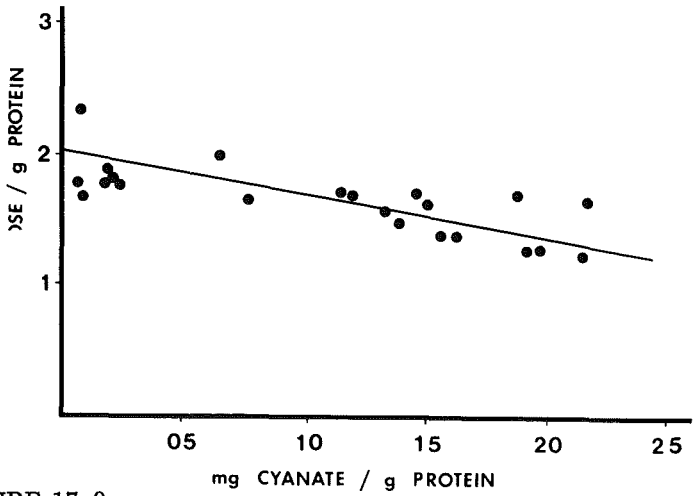

FIGURE 17-9
Graph of degree of glycosylation/degree of carbamylation of serum protein after incubation with 20 mmol/L glucose of samples previously carbamylated with potassium cyanate.

20 mM ^{14}C glucose for six days at 37°C. Incorporation of radioactive glucose was measured, after repetitive washing and centrifugation of the samples to remove all residues of free glucose, and was shown to correlate inversely with the degree of carbamylation achieved (Fig. 17-9). Altogether, it seems likely that chemical modification of serum albumin by cyanate and by glucose occurs at the same site.

RHEUMATOID ARTHRITIS

Several years ago, it was observed that serum from patients with rheumatoid arthritis showed an abnormally[13] high affinity for the binding of acetrizoate. Although this was initially believed to reflect an alteration in the synthesis of albumin in this disease,[24] it was soon established that this plasma protein binding abnormality of rheumatoid arthritis was mainly related to the treatment used. In fact, it was shown that albumin from normal subjects receiving 2.4 g aspirin per day for three weeks binds significantly more acetrizoate than albumin from control individuals, and that the capacity of normal albumin to bind acetrizoate could be enhanced by dialysis against aspirin in vitro.[12] Moreover, the binding of acetrizoate to plasma proteins in patients with rheumatoid arthritis taking 3 to 5 g of aspirin per day significantly decreased when the treatment was changed to an equivalent dose of sodium salicylate.[17]

It is now firmly established that aspirin can acetylate human serum albumin,[16] and that this acetylation has marked effects on the plasma protein binding of other drugs. Thus, it has been shown that human serum albumin acetylated by aspirin has a greater affinity for phenylbutazone (a site I drug) and a lower affinity for flufenamic acid (a site II drug) than control albumin.[7] Interestingly, the changes induced by aspirin are not brought off by other acylating agents, and it has been reported that acetic anhydride, benzylpenicillin, and benzylpenicillenic acid do not alter the binding affinity of human serum albumin for flufenamic acid.[7] This suggests that the specific pattern of acetylation of human serum albumin by aspirin is greatly dependent on the affinity of the protein for this drug. Indeed, it has been reported that salicylate can competitively inhibit the reaction.[23]

In the light of this, the lack of effects of other acylating agents on the drug binding affinity of albumin should perhaps be revised. In the reported experiments, a relatively low concentration (0.5 mM) of aspirin and other agents was used. Benzyl penicillin is used in extremely high intravenous doses in some conditions, and concentrations in blood much higher than 0.5 mM are likely to be reached. This may also happen with some acylating cephalosporins.

OTHER DRUG-INDUCED COVALENT MODIFICATIONS OF HUMAN SERUM ALBUMIN

A chemical modification of human serum albumin induced by galactose in vitro has been described,[32] and, similarly, nonenzymatic galactosylation of albumin in a galactosemic infant has been reported.[33] Although the plasma protein binding of drugs in this condition has not been explored, it is of interest to notice that the finding of this altered albumin in vivo led the authors of the report to speculate that the transport of metabolites, such as bilirubin, might be altered in galactosemia.

Bilirubin should also be considered a possible cause of modification of human serum albumin. Ordinarily, bilirubin is bound to albumin in a manner similar to most drugs, but a possible covalent reaction of albumin with human serum bilirubin has been described.[20,35] The bilirubin binding site on human serum albumin seems clearly independent of the benzodiazepine binding site (site II) but its relation to the warfarin site (site I) is not quite clear.[14] In fact, a decrease in the binding of warfarin induced by bilirubin has been reported,[28] but no displacement interactions between bilirubin and salicylates or sulfonamides are observed when these ligands bind to primary sites.[31] At any event, it seems possible that a

covalent modification of albumin by bilirubin might result in an altered binding of some drugs.

A further example of posttranslational modification of albumin is offered by the use of nitrosoureas as cytotoxic agents. Under physiological conditions, nitrosoureas spontaneously liberate isocyanates that are capable of carbamylation of proteins.[22] In a series of experiments with carmustine, it was shown that this nitrosourea carbamylates plasma proteins in vitro, and that this carbamylation of proteins results in an altered binding of sulfisoxazole with no change, or even a slight increase, in the plasma protein binding of diazepam.[3]

Although their exact quantitative role in the altered transport of drugs in plasma in disease states cannot yet be established, it seems that posttranslational changes in the molecule of albumin should be recognized as a possible cause of altered binding of drugs in plasma. There are examples to show that these changes may be produced either by chemicals associated with the disease or by drugs used in its management, and the search for them is probably worth continuing.

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