Serum Protein Binding Alterations of Selected Cephalosporin Antibiotics by Fatty Acids and Their Derivatives

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Abstract □ Saturated fatty acids containing 10–14 carbon atoms were more potent inhibitors of serum protein binding than those containing shorter or longer carbon chains. Introduction of unsaturation into chains containing 16 or 18 carbons increased their inhibitory potency. Triglycerides and fatty acid esters, chlorides, thiols, and amides had no inhibitory activity. When inhibition was observed, it was concentration dependent and occurred when the molar ratio of fatty acid to protein equaled or exceeded three. The change in percent serum protein binding in the presence of an effective inhibitor was the greatest with cephalosporins that were most highly bound in the absence of an inhibitor.

Keyphrases □ Antibiotics—cephalosporins, serum protein binding, alteration by fatty acids and their derivatives □ Cephalosporins—serum protein binding, alteration by fatty acids and their derivatives □ Protein binding—cephalosporin antibiotics, alteration by fatty acids and their derivatives, serum

Serum protein binding can be diminished in several disease states, after administration of certain hormones, anticoagulants, and sulfa drugs, or by alteration of the levels of normally occurring metabolites such as bilirubin or fatty acids (1–6). Even small changes in serum protein binding can produce clinically significant physiological effects, particularly with drugs that are highly bound. These changes may affect not only the amount of free drug in the plasma but also its tissue concentration, tissue distribution, and elimination pharmacokinetics (1, 5, 7–9).

Kunin (4) showed that the content of free penicillin analogs in humans may be increased by coadministration of sulfamethoxypyridazine or sulfaethidole (sulfaethylthiadiazole), two *in vitro* inhibitors of serum protein binding. Spector (8) stressed the need to determine if the ability of albumin to transport a second compound can be influenced by free fatty acid concentrations in plasma. In this paper, data are presented on the relationship between the structure and serum protein binding inhibition of seven saturated and four unsaturated fatty acids in addition to the effects of chemical substituents on the carboxy terminus.

EXPERIMENTAL

Cephalothin and cefazolin were obtained from commercial sources. Cefonicid sodium [(6*R*,7*R*)-7-[(*R*)-mandelamido]-8-oxo-3-[[[1-(sulfomethyl)-1*H*-tetrazol-5-yl]thio]methyl]-5-thia -1- azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid disodium salt], a new, long-acting parenteral cephalosporin (10), was synthesized[1]. Fatty acids were obtained commercially at a stated purity of >98%, while analogs were obtained at a stated purity of >95%. Fatty acids and their analogs were dissolved at high concentrations in ethanol or ethanol–chloroform, depending on solubility.

Human serum was obtained from a local commercial supplier of blood products. Serum pH was adjusted to 7.2–7.4 with carbon dioxide before use. Small volumes of fatty acid or other stock solutions or the diluent used in their preparation were added in varying concentrations to the serum, which was then held at 4° for 16–18 hr before use. This storage permitted the additive to interact with the serum proteins (9, 11). The additives to the serum were present at molar ratios of fatty acid to serum protein of 0:1, 1:1, 3:1, 5:1, 7.5:1, or 10:1, whereas the fatty acid analogs were tested at ratios of 0:1, 3:1, and 10:1 (5, 12, 13).

Samples were assayed using a disk agar diffusion test employing *Bacillus subtilis* (ATCC 6633) grown in penicillin assay seed agar as the indicator. Corrections were made for intergroup variation in the indicator response by assaying an internal standard. Serum protein binding determinations were made using the standard response line offset technique. In this technique, the response of the indicator to five concentrations of the selected antibiotic in 1% pH 7.4 phosphate buffer was compared to the response produced when the antibiotic was diluted in serum. The concentration of antibiotic in serum, S, producing a specified response was compared to the concentration of antibiotic in buffer, B, producing the identical response. The value $(1 - B)/S \times 100$ was computed and reported as the percent serum protein binding (11, 14).

RESULTS

The three antibiotics used, cephalothin, cefazolin, and cefonicid, were chosen in control experiments on the basis of their serum protein binding as being representatives of moderately, highly, and very highly bound cephalosporins.

The effects of adding C_6–C_{18}-saturated fatty acids at various fatty acid to protein molar ratios on the serum protein binding of the three cephalosporins are shown in Figs. 1–3. The data show the difference in percent serum protein binding with and without an inhibitor in the serum as a function of inhibitor chain length. The amount of inhibition was dependent on fatty acid concentrations and occurred at ratios of 3:1 or greater. The various fatty acids differed in their ability to inhibit serum protein binding. With cephalothin, maximal inhibition occurred at C_{10} (Fig. 1); it occurred at C_{10}–C_{12} (Fig. 2) for cefazolin and at C_{12} for cefonicid (Fig. 3).

Results obtained using the standard curve offset technique were confirmed using ultrafiltration techniques and radiolabeled cefonicid with intermediate or high concentrations of a C_{10}-fatty acid as a serum additive. These results indicate that data obtained using the standard curve offset technique are not attributable to artifacts introduced by the assay system. Fatty acids exerted their greatest effect on cephalosporins that were most highly bound in the absence of an inhibitor. The carbon chain length requirements for inhibition of cefazolin appear to be slightly less

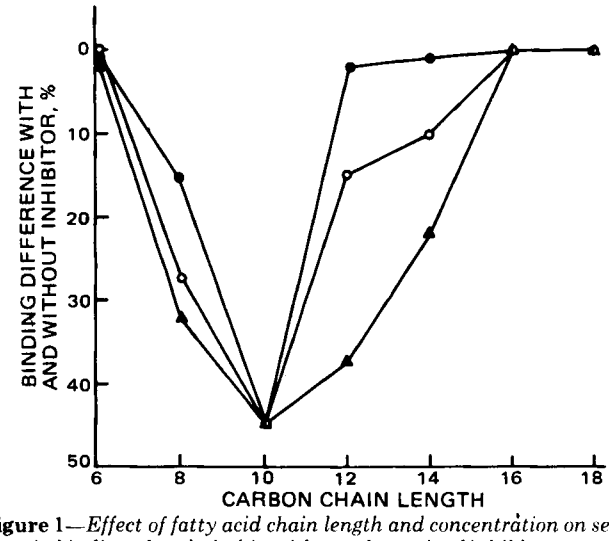

Figure 1—*Effect of fatty acid chain length and concentration on serum protein binding of cephalothin with a molar ratio of inhibitor to protein of 3:1 (●), 5:1 (○), and 10:1 (△).*

[1] Smith Kline & French Laboratories.

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Table I—Effect of Fatty Acid Unsaturation on Serum Protein Binding of Three Cephalosporins at a Fatty Acid to Protein Ratio of 10:1[a]

Fatty Acid	Cephalothin	Cefazolin	Cefonicid
Palmitic (16[b])	0	34	23
Palmitoleic (16:1)	38	50	83
Stearic (18)	0	9	7
Oleic (18:1)	25	42	61
Linoleic (18:2)	14	54	68
Linolenic (18:3)	41	47	69

[a] Data are shown as a change in the percent bound from the control. [b] Number of carbons to unsaturated bonds.

stringent than those for inhibition of cephalothin or cefonicid. In all cases, adding a potential inhibitor at inhibitor to serum protein ratios of 1:1 failed to affect the serum protein binding significantly.

The effect of structural modification of saturated fatty acids on their ability to act as serum protein binding inhibitors was examined. Fatty acids having carbon chain lengths of 16 (poorly effective) or 18 (ineffective) were used as examples. Introduction of monounsaturation or multiple unsaturation into these compounds profoundly changed their potency as serum protein binding inhibitors (Table I).

The next structural modification examined was substitution at the carboxy terminus. Fatty acids with a 12 carbon chain (lauric acid) and a monounsaturated 18 carbon chain (oleic acid) were studied. The results of substituting the carboxy group with sodium, alcohol, amide, chloride, methyl ester, or thiol groups or of using the triglyceride on the serum protein binding of the three cephalosporins are shown in Table II. These tests were carried out with a 10:1 inhibitor to protein ratio. Only the acid and its sodium salt were effective serum protein inhibitors.

DISCUSSION

These results show that saturated fatty acids having 10–14 carbon atom chains markedly inhibited serum protein binding of all tested cephalosporins whereas fatty acids with shorter or longer carbon chains were less effective. Introduction of unsaturation or substitution at the carboxy terminus also altered their potency. This effect suggests that the interaction is structure dependent and possibly is due to the degree of lipophilicity of the inhibitor. This idea is supported by the findings that introduction of unsaturation into 16–18 carbon atom chains markedly increase their inhibitory ability while substitution at the carboxy terminus with any of several negatively charged groups or esterification in the presence or absence of glycerol eliminated the serum protein binding inhibitory effect.

These findings are in agreement with earlier work (8) which indicated that the bond between fatty acids and human serum albumin was due

Table II—Effect of Chemical Substitution at the Carboxy Terminus on the Serum Protein Binding Inhibitory Potency of C_{12} or Monounsaturated C_{18} Fatty Acids

Inhibitor	Cephalothin	Cefazolin	Cefonicid
Lauric acid[a]	37	47	64
Sodium salt	33	44	64
Alcohol	0	4	4
Chloride	0	0	0
Methyl ester	0	4	0
Thiol	0	0	0
Triglyceride	5	0	0
Oleic acid[b]	33	41	61
Sodium salt	33	48	77
Alcohol	0	2	1
Chloride	0	7	2
Amide[c]	0	0	0
Methyl ester	0	1	0
Triglyceride	3	0	1

[a] Twelve carbon chain. [b] Eighteen carbon chain, with one unsaturated bond. [c] Tested at a ratio of 5:1 because of inhibition of the assay organism at a ratio of 10:1.

predominantly to lipophilic rather than electrostatic or hydrogen bond interactions. Fatty acid esters or triglycerides were not inhibitory (8). The findings of Vallner (12) and Rudman *et al.* (5) were verified in that maximal inhibitory effects were observed when the fatty acid to protein ratio was three or greater. Previous findings that highly bound drugs show a more pronounced response than drugs with lower binding also were confirmed (1).

In related studies, probenecid and novobiocin, both organic acids that are highly serum protein bound, also were effective *in vitro* inhibitors of serum protein binding using the three cephalosporins. These findings suggest that variation in the fatty acid content of human serum or coadministration of other highly serum protein bound compounds have the potential to affect dramatically the serum protein binding of commonly used antibiotics.

Changes in serum protein binding can be important clinically with regard to potential toxicity of highly bound drugs having a narrow therapeutic ratio. In addition, alterations in serum protein binding may affect

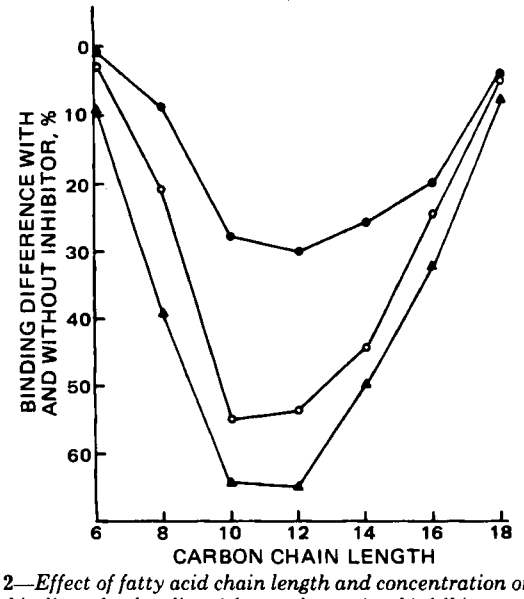

Figure 2—*Effect of fatty acid chain length and concentration on serum protein binding of cefazolin with a molar ratio of inhibitor to protein of 3:1 (●), 5:1 (○), and 10:1 (△).*

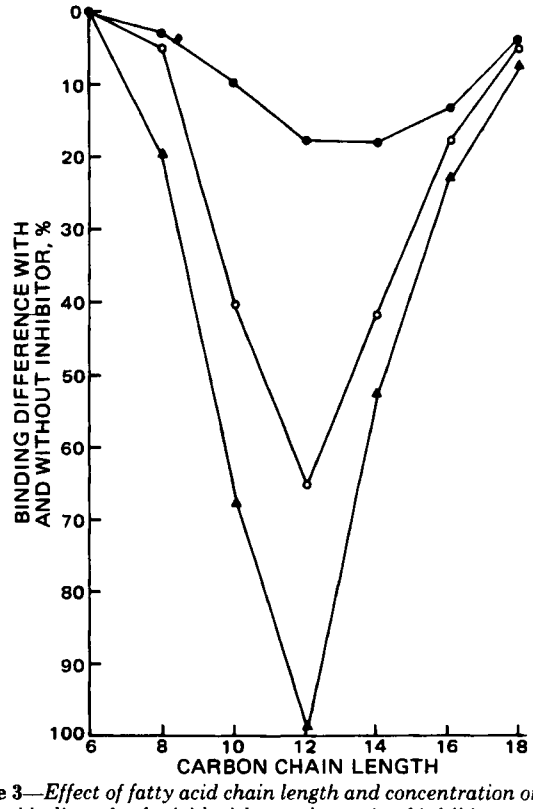

Figure 3—*Effect of fatty acid chain length and concentration on serum protein binding of cefonicid with a molar ratio of inhibitor to protein of 3:1 (●), 5:1 (○), and 10:1 (△).*

the bioassay of samples containing multiple drugs or abnormal levels of normally occurring metabolic products.

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Absorption of Sodium γ-Hydroxybutyrate and Its Prodrug γ-Butyrolactone: Relationship between *In Vitro* Transport and *In Vivo* Absorption

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Received August 20, 1979, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication October 11, 1979.

Abstract □ A qualitative relationship between *in vitro* transport and *in vivo* absorption of sodium γ-hydroxybutyrate and γ-butyrolactone was demonstrated. As with other short-chain acids, sodium γ-hydroxybutyrate showed capacity-limited transport *in vitro*, consistent with the previous observation that this drug exhibited slower *in vivo* absorption with increasing dose. The prodrug lactone, on the other hand, showed a higher intestinal flux than the acid in the everted gut, and *in vivo* absorption also was more rapid. Capacity-limited transport and absorption of the lactone appeared less evident. Thus, the increased oral hypnotic activity of the lactone over that of the acid most likely is a result of its more favorable intestinal transport characteristics.

Keyphrases □ Sodium γ-hydroxybutyrate—relationship between *in vitro* transport and *in vivo* absorption □ γ-Butyrolactone—prodrug for sodium γ-hydroxybutyrate, relationship between *in vitro* transport and *in vivo* absorption □ Hypnotic agents—sodium γ-hydroxybutyrate and γ-butyrolactone, relationship between *in vitro* transport and *in vivo* absorption

γ-Hydroxybutyrate (I), a metabolite of γ-aminobutyric acid, is found endogenously in the human brain (1). When introduced intravenously, I is a useful anesthetic (2) and is beneficial in Parkinson's disease (3). However, oral administration of this compound results in decreased and variable pharmacological activity (4–6). Recently, oral doses of I totaling 50 mg/kg were shown to be useful in the treatment of narcolepsy and cataplexy in patients, but the duration of sleep induction after each oral dose lasted only for ~2 hr (7).

BACKGROUND

In previous animal studies in these laboratories (8–10), orally administered I was shown to be subject to first-pass metabolism at low doses (≤200 mg/kg) in rats. At higher doses (400–1600 mg/kg), systemic availability approached 100%, presumably due to saturation of first-pass metabolism, but the relative absorption rate appeared to decrease with increasing dose. Thus, although the extent of drug absorption was almost complete, peak plasma I concentrations were relatively insensitive to increases in the oral dose and, in most animals, threshold hypnotic concentrations in plasma were not reached in spite of high oral doses.

The lactone analog of I, γ-butyrolactone (II), is hydrolyzed rapidly and exclusively *in vivo* to I (11, 12) and, therefore, can be classified as a prodrug. Compound II is rapidly and completely absorbed *in vivo* after oral administration over a wide dose range. In contrast to I, the peak drug concentration after oral dosing of II was proportional to the dose, and II was equally effective as a hypnotic whether given orally or intravenously (9).

The reason for the apparent difference in *in vivo* absorption characteristics between I and II has not been delineated. In this paper, *in vitro* experiments that compared the transport properties of these two compounds across the everted rat gut are described.

EXPERIMENTAL

Reagents—Compound I, obtained as the sodium salt[1], and II[1] were used without purification. The buffer and assay reagents[1–3] were all reagent or analytical grade.

Everted Rat Gut Preparation—Male Sprague–Dawley rats, 260–310 g, were sacrificed by decapitation. An intestinal segment, ~12 cm long, was taken from a region 20 cm from the pylorus sphincter; it was everted and mounted according to the technique originally devised by Wilson and Wiseman (13) and modified by Crane and Wilson (14).

Flux Experiment—The everted gut was placed inside a test tube with the mucosal side exposed to 90 ml of a 0.05 *M* physiological tromethamine buffer (pH 7.4) containing the appropriate drug concentration. All flux studies were carried out at 37°. At 5-min intervals up to 25 min, the serosal solution (~1 ml) was removed for the assay and replaced with an equal volume of fresh buffer. Three or four replicate flux experiments were conducted at each initial mucosal concentration.

Spectrophotometric Analysis—The Hestrin (15) assay for short-chain *O*-acyl derivatives as adopted for I and II by Guidotti and Ballotti (16) was employed. Conversion of I to II was effected by reaction with two parts of concentrated sulfuric acid[2] and subsequent neutralization with 10 parts of 6 *N* NaOH[2].

[1] Eastman Kodak Co., Rochester, NY 14650.
[2] Fisher Scientific Co., Fair Lawn, NJ 07410.
[3] J. T. Baker Chemical Co., Phillipsburg, NJ 08865.

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