Binding of Fluvastatin to Blood Cells and Plasma Proteins

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Abstract □ The binding of fluvastatin, an inhibitor of hydroxymethylglutaryl coenzyme A reductase, to plasma proteins and red blood cells of rat, dog, and human in vitro was determined by ultrafiltration. Additionally, the stereospecificity of fluvastatin binding to proteins and the potential interaction between fluvastatin and the highly protein bound drugs warfarin, salicylic acid, and glyburide were investigated. Only a small fraction of fluvastatin in blood was taken up by the blood cells, amounting to 19–33% in the rat and ≤15% in dog and humans. The plasma:blood fluvastatin ratio in these species at 37 °C was ≥1.4. In human blood, this ratio was temperature independent. In the plasma concentration range 25–50 000 ng/mL, fluvastatin was ≥98% bound to proteins. The binding was concentration dependent in the rat, but not in the dog and human. Both enantiomers of fluvastatin were >99% bound in normal human plasma, the binding of each being unaffected by the presence of the other. A major fluvastatin-binding protein in human plasma was albumin, whereas binding to α_1-acid glycoprotein was relatively weak and concentration dependent. At therapeutic concentrations in normal human plasma, the protein binding of fluvastatin (0.1 μg/mL) was unaffected by warfarin (1–10 μg/mL), salicylic acid (50–150 μg/mL), and glyburide (0.1–1 μg/mL). Similarly, fluvastatin had no influence on the binding of these compounds. In diluted human albumin solution (29 μM), bound fluvastatin was displaced by all three co-solutes tested. The total binding constant of fluvastatin was reduced by ~40%, 50%, and sevenfold in the presence of warfarin, glyburide, and salicylic acid, respectively. Conversely, fluvastatin reduced the total binding constant of salicylic acid by ~34% but had little effect on the binding of warfarin and glyburide. The mutual inhibition between fluvastatin and salicylic acid appears to be of the competitive type.

Fluvastatin (Sandoz compound XU 62-320; **1**; [*R**,*S**-(*E*)]-(±)-sodium-3,5-dihydroxy-7-[3-(4-fluorophenyl)-1-(1-methylethyl)-1H-indole-2yl]-hept-6-enoate) is a potent inhibitor of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthesis.[1,2] Previous studies have shown significant reduction in serum total cholesterol, low-density lipoprotein cholesterol, and serum triglyceride levels in rats, dogs, and monkeys treated with fluvastatin.[3] The involvement of low-density lipoprotein (LDL) cholesterol in atherogenesis has been well documented.[4] Plasma levels of LDL cholesterol are now recognized to be causally related to the development of coronary heart disease.[5]

Fluvastatin has two asymmetric carbons, the 3- and 5-carbons of the heptenoic acid side chain, which results in two possible diastereomers, *erythro* and *threo*. The cholesterol-lowering agent under investigation has the *erythro* configuration and is a racemic mixture of two of four possible stereoisomers, 3*R*,5*S* (Sandoz compound 262-735, **2**) and 3*S*,5*R* (Sandoz compound 262-850, **3**). The pharmacokinetic properties of the racemate have been studied in a number of laboratory species[6,7] and in humans.[8]

The present report concerns the binding of fluvastatin to plasma proteins and blood cells of the rat, dog, and human in the concentration range encompassing the peak and trough levels experienced in these species. Binding of drugs to plasma proteins has long been recognized as having an important effect on the duration and intensity of drug action.[9,10] Because the unbound drug in plasma is considered to account for the pharmacologic activity of the drug,[11] the displacement of a drug from its protein binding sites by another can be the mechanism of significant drug–drug interactions.[12] In the present study, the competitive binding of fluvastatin to plasma proteins with the potentially coadministered drugs warfarin, salicylic acid, and glyburide was investigated. Furthermore, the stereospecificity of binding of fluvastatin to proteins was examined.

Experimental Section

Drug Substances—^{3}H-Labeled fluvastatin (Isotope Laboratory, Sandoz Research Institute, East Hanover, NJ; lot nos 1239-203-39 and 1408-4-30), **2** (Sandoz, lot no 1408-17-34), and **3** (Sandoz, lot no 1408-11-36) were synthesized with the tritium label introduced specifically at the 3-position of the *p*-fluorophenyl moiety (see structure). The radiochemical purity of each product was >95%, and the specific activities ranged from 131 to 180 μCi/mg. ^{14}C-Labeled warfarin (Amersham, Arlington Heights, IL; lot no 40; radiochemical purity, 98.8%; specific activity, 181 μCi/mg) and salicylic acid (ICN Radiochemicals, Irvine, CA; lot no 3339143; radiochemical purity, >99%; specific activity, 404 μCi/mg), as well as nonradiolabeled warfarin (Aldrich, Milwaukee, WI; lot no 18504LX) and salicylic acid (Aldrich, lot no 04726CX) were obtained commercially. [^{14}C]Glyburide (lot no CSL-89-241-79-01; radiochemical purity, 96.4%; specific activity, 105 μCi/mg) and nonradiolabeled glyburide (lot no 964DD) were gifts from The Upjohn Company (Kalamazoo, MI).

Blood, Plasma, and Protein Solutions—Fresh, heparinized blood was obtained from Sprague-Dawley rats, beagle dogs, and two human volunteers who gave written informed consent. Plasma was separated by centrifugation at ~1000 × *g* for 15 min. Commercially available human serum albumin (HSA; Fraction V; Sigma, St. Louis, MO; lot no 107F-9346) and α_1-acid glycoprotein (AAG; purified from Cohn Fraction VI; Sigma; lot no 36F-9390) were used to prepare protein solutions in 0.02 M phosphate buffer (pH 7.4).

Sample Preparation—Solutions of the radiolabeled and nonradiolabeled drugs were prepared in the appropriate matrix, and serial dilutions were performed to yield the desired test concentrations of each solute (Table I). Control experiments were conducted for the maximum concentration range of each drug in 0.02 M phosphate buffer (pH 7.4). In this report, the concentrations of fluvastatin, **2**, and **3** are expressed as the equivalents of the free acid.

Binding with Red Blood Cells—Quadruplicate determinations of the hematocrit were made for each species. Aliquots of blood containing [^{3}H]fluvastatin were pipetted for radioactivity analysis. The remaining blood was centrifuged (~1000 × *g*) at 37 °C for 15 min, and the resultant plasma was analyzed in triplicate for radioactivity. For human blood samples only, the experiment was repeated at 25 and 0 °C.

Ultrafiltration—Aliquots of the drug solutions in phosphate buffer (controls), plasma, physiologic HSA (6.5 × 10^{-4} M), and AAG (1.7 × 10^{-5} M), as well as diluted HSA (2.9 × 10^{-5} M), were pipetted in

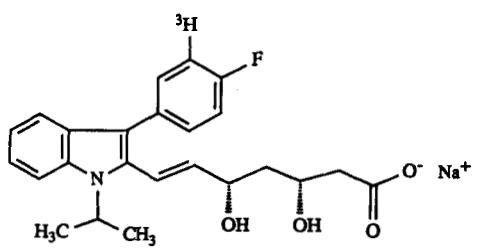

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Table I—Drug Solutions and Concentrations Tested

Radiolabeled Drug	Matrix	Concentration, μg/mL	Co-solute	Concentration, μg/mL
[^{3}H]Fluvastatin	Blood and plasma from rat, dog, and humans; HSA (650 μM); AAG (17 μM)	0.025, 0.05, 0.1, 0.5, 1, 5, 25, 50	—[a]	—
[^{3}H]Fluvastatin	Human plasma	0.1	—	—
			Warfarin	1, 10
			Salicylic acid	50, 150
			Glyburide	0.1, 1
	HSA (29 μM)	0.05, 0.1, 0.5, 1.5	—	—
			Warfarin	1, 10
			Salicylic acid	50, 150
			Glyburide	0.1, 1
[^{14}C]Warfarin	Human plasma	1, 10	—	—
			Fluvastatin	0.1
	HSA (29 μM)	1, 5, 10, 50, 100, 500	—	—
			Fluvastatin	0.1
[^{14}C]Salicylic acid	Human plasma	50, 150	—	—
			Fluvastatin	0.1
	HSA (29 μM)	1, 5, 10, 50, 100, 150, 500	—	—
			Fluvastatin	0.1
[^{14}C]Glyburide	Human plasma	0.1, 0.2, 1	—	—
			Fluvastatin	0.1
	HSA (29 μM)	0.1, 0.2, 1, 2, 10, 20	—	—
			Fluvastatin	0.1
[^{3}H]**2**	Human plasma	0.1	—	—
			3	0.1
[^{3}H]**3**	Human plasma	0.1	—	—
			2	0.1

[a] —, No co-solute.

triplicate for radioactivity analysis. Subsequently, three aliquots (1 mL) of each sample were transferred to the sample reservoir of individual micropartition centrifuge tubes (Centrifree Micropartition Centrifuge Tube, Amicon, Beverly, MA). The tubes were capped and maintained at 37 °C until and during centrifugation in a refrigerated centrifuge (Sorvall RC-5B Superspeed Refrigerated Centrifuge, DuPont, Wilmington, DE) for 5 min at ~1500 × g. An aliquot of each ultrafiltrate was analyzed for radioactivity.

Analysis of Radioactivity—Radioactivity was measured in a liquid scintillation spectrometer (Packard Tri-Carb, model 460, Packard, Downers Grove, IL). For the binding study with red blood cells, blood and plasma aliquots (0.2 mL) were air dried and combusted in an oxidizer (Packard Tri-Carb, model 306, Packard) before counting in a liquid scintillant (Monophase S, Packard). For studying binding with plasma proteins, aliquots (0.1 mL) of plasma, protein solutions, and the ultrafiltrates were mixed with 10 mL of a liquid scintillant (Formula-989, DuPont, Wilmington, DE, or ACS, Amersham, Arlington Heights, IL) in a vial for direct counting.

Data Interpretation—The fraction of drug in blood that is distributed to red blood cells (f_{BC}) is calculated according to eq 1:

$$f_{BC} = 1 - [(1 - H) \cdot (C_p/C_b)] \quad (1)$$

In eq 1, H is the hematocrit and C_p and C_b are the concentrations of radioactivity in plasma and blood, respectively.[13] The fraction of drug bound to plasma proteins (β) equals $(T - U)/T$, where T is the concentration of total radioactivity in the uncentrifuged sample and U is the concentration of radioactivity in the ultrafiltrate. Control experiments revealed negligible binding (<1.5%) of warfarin, salicylic acid, and glyburide to the membrane. However, the fraction of drug lost (L) due to binding to the centrifuge tube and/or membrane was significant for fluvastatin, 2, and 3. For these compounds, β was corrected as follows:

$$\beta = [T - U/(1 - L)]/T \quad (2)$$

The nature of the drug–albumin interactions was analyzed by Scatchard plots.[9] For a drug that binds to a single set of binding sites, the molar concentrations of bound drug (C_B) and free drug (C_F) are related by the following equation:

$$C_B/C_F = k \cdot n \cdot P - k \cdot C_B \quad (3)$$

In eq 3 n is the number of binding sites, P is the molar concentration of albumin, and k is the intrinsic association constant. These parameters were readily obtained by linear regression analysis of the plot C_B/C_F versus C_B. The total binding constant (K) equals $n \cdot k$.

For a drug that is associated with two sets of binding sites:

$$C_B/C_F = P(Z_1 + Z_2 + Z_3)/(1 + Z_3 \cdot C_F + Z_4 \cdot C_F^2) \quad (4)$$

In eq 4, $Z_i = n_1 \cdot k_1 + n_2 \cdot k_2$, $Z_2 = (n_1 + n_2) \cdot k_1 \cdot k_2$, $Z_3 = k_1 + k_2$, $Z_4 = k_1 \cdot k_2$, and n_1, n_2 and k_1, k_2 are the numbers and association constants pertaining to the respective sets of binding sites.[14] Data were fitted to the curve by nonlinear least-squares fit methods with the program NONLIN.[15] Statistical comparisons were made with the t-test for data analysis. Results are statistically different at a significance level of $p < 0.05$.

Results and Discussion

Distribution in Blood—In rat, dog, and human blood with hematocrit values of 0.52 ± 0.02 (mean ± SD), 0.49 ± 0.01, and 0.49 ± 0, respectively, the bulk of fluvastatin was present in the plasma component. As shown in Table II, the plasma:blood ratio (C_p/C_b) in all three species at 37 °C was ≥1.40 over the blood concentration range 25–50 000 ng/mL. In the rat, C_p/C_b progressively declined with increases in concentration from 1.69 at 25 ng/mL to 1.40 at 50 000 ng/mL, whereas the fraction distributed to blood cells (f_{BC}) increased from 0.19 to 0.33. In dog and human, the distribution of fluvastatin in blood appeared to be independent of the total blood concentration. Both species showed a greater amount of drug in plasma than the rat, with C_p/C_b values of 1.66–2.11 and f_{BC} of 0–0.15. The C_p/C_b values in human blood measured at 25 and 0 °C were similar to those at 37 °C (Table III), suggesting temperature independence of this parameter under the testing conditions.

Binding to Plasma Proteins—The extent of binding of

Table II—Plasma:Blood Ratio (C_p/C_b) and Fraction of [^{3}H]Fluvastatin Distributed to Red Blood Cells (f_{BC}) at 37 °C

Blood Concentration, ng/mL	C_p/C_b			f_{BC}		
	Rat	Dog	Human	Rat	Dog	Human
25	1.69	1.83	1.93	0.19	0.07	0.02
50	1.66	1.92	1.83	0.20	0.02	0.07
100	1.61	1.80	1.81	0.23	0.08	0.08
500	1.52	1.75	1.72	0.27	0.11	0.12
1 000	1.54	1.66	1.78	0.26	0.15	0.09
5 000	1.45	1.70	1.77	0.30	0.13	0.10
25 000	1.41	1.75	1.83	0.32	0.11	0.07
50 000	1.40	1.74	2.11	0.33	0.11	0

Table III—Plasma:Blood Ratio (C_p/C_b) of [^{3}H]Fluvastatin in Human Blood as a Function of Temperature

Blood Concentration, ng/mL	C_p/C_b		
	37 °C	25 °C	0 °C
25	1.93	1.79	1.79
50	1.83	1.82	1.70
100	1.81	1.86	1.67
500	1.72	1.78	1.66

Table IV—Fraction of [^{3}H]Fluvastatin Bound to Plasma Proteins (β)

Plasma Concentration, ng/mL	β[a]		
	Rat	Dog	Human
25	1.0000 ± 0	0.9887 ± 0.0023	0.9974 ± 0.0024
50	1.0000 ± 0	0.9881 ± 0.0011	0.9946 ± 0.0017
100	0.9974 ± 0.0002	0.9905 ± 0.0011	0.9926 ± 0.0005
500	0.9871 ± 0.0010	0.9911 ± 0.0004	0.9916 ± 0.0008
1 000	0.9857 ± 0.0007	0.9916 ± 0.0012	0.9899 ± 0.0004
5 000	0.9818 ± 0.0006	0.9892 ± 0.0016	0.9903 ± 0.0011
25 000	0.9790 ± 0.0005	0.9890 ± 0.0004	0.9903 ± 0.0003
50 000	0.9805 ± 0.0007	0.9886 ± 0.0003	0.9900 ± 0.0016

[a] Values are mean ± SD; n = 3.

Table V—Fraction of [^{3}H]Fluvastatin Bound (β) to HSA and α_1-Acid Glycoprotein

[^{3}H]Fluvastatin Concentration, ng/mL	β[a]	
	Albumin	α_1-Acid Glycoprotein
25	1.0000 ± 0	0.6702 ± 0.0313
50	1.0000 ± 0	0.6370 ± 0.0255
100	0.9983 ± 0.0007	0.6132 ± 0.0641
500	0.9949 ± 0.0008	0.6715 ± 0.0157
1 000	0.9939 ± 0.0008	0.5708 ± 0.0168
5 000	0.9933 ± 0.0010	0.3972 ± 0.0625
25 000	0.9939 ± 0.0003	0.1327 ± 0.0300
50 000	0.9939 ± 0.0002	0.0617 ± 0.0077

[a] Values are mean ± SD; n = 3.

Table VI—Binding of [^{3}H]Fluvastatin Enantiomers to Human Plasma Proteins

Test Compound[a]	Co-solute	β[b]
[^{3}H]**2**	—[c]	0.9916 ± 0.0011
[^{3}H]**2**	**3**	0.9910 ± 0.0004
[^{3}H]**3**	—	0.9930 ± 0.0005
[^{3}H]**3**	**2**	0.9926 ± 0.0006

[a] Plasma concentrations of both test compound and co-solute were 0.1 μg/mL. [b] Values are mean ± SD; n = 3. [c] —, No co-solute.

Table VII—Fraction of Drug Bound to Human Plasma Proteins (β)

Test Compound	Test Compound Concenration, μg/mL	Co-Solute	Co-Solute Concenration, μg/mL	β[a]
[^{3}H]Fluvastatin	0.1	—[b]		0.9910 ± 0.0011
		Warfarin	1	0.9913 ± 0.0014
			10	0.9922 ± 0.0006
		Salicylic Acid	50	0.9917 ± 0.0006
			150	0.9914 ± 0.0005
		Glyburide	0.1	0.9917 ± 0.0019
			1	0.9918 ± 0.0010
[^{14}C]Warfarin	1	—		0.9445 ± 0.0151
		Fluvastatin	0.1	0.9694 ± 0.0060
	10	—		0.9830 ± 0.0020
		Fluvastatin	0.1	0.9855 ± 0.0012
[^{14}C]Salicylic acid	50	—		0.9018 ± 0.0180
		Fluvastatin	0.1	0.9060 ± 0.0024
	150	—		0.8650 ± 0.0110
		Fluvastatin	0.1	0.8634 ± 0.0035
[^{14}C]Glyburide	0.1	—		0.9822 ± 0.0016
		Fluvastatin	0.1	0.9817 ± 0.0021
	0.2	—		0.9802 ± 0.0018
		Fluvastatin	0.1	0.9810 ± 0.0023
	1	—		0.9818 ± 0.0016
		Fluvastatin	0.1	0.9820 ± 0.0010

[a] Values are mean ± SD; n = 3. [b] —, No co-solute.

Table VIII—Binding of [^{3}H]Fluvastatin in Diluted HSA Solution (29 μM) With or Without Warfarin, Salicylic Acid, and Glyburide

[^{3}H]Fluvastatin Concentration, μg/mL	β[a]						
	Alone	Warfarin, μg/mL		Salicylic Acid, μg/mL		Glyburide, μg/mL	
		1	10	50	150	0.1	1
0.05	0.9753 ± 0.0059	0.9440 ± 0.0055[b]	0.9600 ± 0.0037[b,c]	0.9124 ± 0.0147[b]	0.8652 ± 0.0177[b,c]	0.9477 ± 0.0041[b]	0.9520 ± 0.0081[b]
0.1	0.9726 ± 0.0024	0.9657 ± 0.0033[b]	0.9547 ± 0.0043[b,c]	0.9155 ± 0.0115[b]	0.8351 ± 0.0544[b,d]	0.9629 ± 0.0046[b]	0.9496 ± 0.0040[b,c]
0.5	0.9766 ± 0.0032	0.9637 ± 0.0072[b]	0.9618 ± 0.0034[b]	0.8956 ± 0.0244[b]	0.8312 ± 0.0427[b,d]	0.9632 ± 0.0062[b]	0.9556 ± 0.0036[b]
1	0.9703 ± 0.0040	0.9445 ± 0.0141[b]	0.9534 ± 0.0100	0.9206 ± 0.0150[b]	0.8374 ± 0.0562[b,d]	0.9242 ± 0.0111[b]	0.9452 ± 0.0057[b,c]
5	0.9630 ± 0.0057	0.8957 ± 0.0143[b]	0.9299 ± 0.0094[b,c]	0.8279 ± 0.0085[b]	0.7160 ± 0.0714[b,d]	0.9299 ± 0.0027[b]	0.9214 ± 0.0116[b]

[a] Values are mean ± SD; n = 3. [b] Significantly different from control at $p < 0.05$. [c] Significantly different from results at lower co-solute concentration ($p < 0.05$). [d] Difference from results at lower co-solute concentration was of borderline significance ($0.05 < p < 0.1$).

fluvastatin to plasma proteins is summarized in Table IV. No appreciable differences were found between rat, dog, and human plasma, all of which indicated a high degree of binding ($\beta \geq 0.98$). Whereas the rat data showed a slight concentration dependency over the wide range of concentrations tested, both dog and human demonstrated a virtually constant degree of binding. This difference in binding capacity between species probably can be explained by a lower plasma concentration of albumin, the main drug-binding protein, in the rat than in the dog or human.[16,17] The extensive binding of fluvastatin to plasma proteins is similar to that reported for other inhibitors of HMG-CoA reductase, including lovastatin[18] and simvastatin.[19] As shown in Table V, the binding of fluvastatin to human albumin was virtually identical to that observed for total plasma proteins, with $\beta > 0.99$ throughout the 25–50 000-ng/mL concentration range. In contrast, the fraction of fluvastatin bound to α_1-acid glycoprotein decreased with increasing drug concentration, with mean values ranging from 0.67 at 25 ng/mL to 0.06 at 50 000 ng/mL. In plasma, both stereoisomers 2 and 3 were bound to virtually the same extent, with <1% of the total concentration unbound (Table VI). The degree of binding was unaffected by the presence of one another at equal, therapeutic levels. The relevance of this finding is supported by preliminary data in humans showing that the concentration ratio of 2 and 3 in vivo is ~1.

Drug–Drug Interactions in Plasma—As shown in Table VII, fluvastatin at a concentration of 0.1 μg/mL was 99.1–99.2% protein bound in human plasma. The bound fluvastatin was not displaced by warfarin, salicylic acid, or glyburide at the tested concentrations. Similarly, the binding of these compounds to plasma proteins was unaffected by the presence of fluvastatin.

Drug Binding in Diluted HSA Solution (29 μM)—The HSA solution was prepared to yield a concentration ~22 times more dilute than the normal serum level of albumin (i.e., ~650 μM[21]). In this solution, fluvastatin alone was 97.5% bound at 0.05 μg/mL, decreasing only slightly to 96.3% at 5 μg/mL (Table VIII). Both warfarin and glyburide showed a consistent but slight displacing effect on binding of fluvastatin to albumin, increasing the unbound fraction of fluvastatin by approximately twofold. However, this effect appeared to be independent of the concentration of warfarin and glyburide tested. In contrast, salicylic acid caused a more marked displacement of fluvastatin, and the effect increased with increasing salicylic acid concentration. As shown in Table IX, the binding of warfarin, salicylic acid, and glyburide to HSA was saturated at elevated concentrations. The presence of fluvastatin had no definitive effect on warfarin or glyburide binding, but tended to reduce the fraction of salicylic acid bound.

To examine the nature of the observed drug–drug interactions, molar concentrations of bound (C_B) and free (C_F) drug were calculated and used to construct Scatchard plots (Figures 1 and 2), from which drug–albumin binding parameters were derived (Table X). The results indicate that a single set of binding sites each was associated with fluvastatin and glyburide in the concentration ranges tested, whereas two sets of binding sites were involved in warfarin and salicylic acid binding to albumin. In diluted HSA solution (29 μM), fluvastatin was bound to one site with an association constant of $1.13 \times 10^6\ M^{-1}$. The calculated binding parameters for the warfarin/HSA,[22,23] salicylic acid/HSA,[24] and glyburide/HSA[25] complexes were in good agreement with those reported previously, although there was considerable variability between studies in the binding parameters for the secondary sites. The total binding constant of fluvastatin was reduced by ~40% in the presence of warfarin, 50% with glyburide, and almost sevenfold with salicylic acid. Conversely, salicylic acid was displaced by fluvastatin from albumin binding sites, the total binding constant being decreased by ~34%. The mutual inhibition between fluvastatin and salicylic acid appears to be of the competitive type because the number of binding sites [~1 for both fluvastatin and salicylic acid (primary site)] remained constant. Therefore, it appears that fluvastatin, like salicylic acid, is mainly bound to site I of HSA (or warfarin site).[26,27] The lack of displacement of warfarin and glyburide, which are known to bind to both warfarin and diazepam (site II) sites,[27] by fluvastatin in the present study

Table IX—Binding of [^{14}C]Warfarin, [^{14}C]Salicylic Acid, and [^{14}C]Glyburide in Diluted HSA Solution (29 μM) With or Without Fluvastatin

Test Compound	Test Compound Concentration, μg/mL	β[a]	
		Fluvastatin, μg/mL	
		0	0.1
[^{14}C]Warfarin	1	0.8730 ± 0.0147	0.8950 ± 0.0129
	5	0.8299 ± 0.0109	0.8424 ± 0.0222
	10	0.7086 ± 0.0206	0.7596 ± 0.0133[b]
	50	0.3588 ± 0.0181	0.4063 ± 0.0452
	100	0.2658 ± 0.0211	0.2349 ± 0.0048
	500	0.0933 ± 0.0079	0.0916 ± 0.0152
[^{14}C]Salicylic acid	1	0.7487 ± 0.0348	0.6638 ± 0.0445[c]
	5	0.6041 ± 0.0781	0.5489 ± 0.0723
	10	0.5191 ± 0.0390	0.4277 ± 0.0141[b]
	50	0.2040 ± 0.0179	0.1807 ± 0.0054[c]
	100	0.1531 ± 0.0130	0.1162 ± 0.0011[b]
	150	0.0968 ± 0.0157	0.0961 ± 0.0173
	500	0.0529 ± 0.0086	0.0451 ± 0.0072
[^{14}C]Glyburide	0.1	0.9564 ± 0.0035	0.9485 ± 0.0083
	0.2	0.9578 ± 0.0052	0.9539 ± 0.0040
	1	0.9567 ± 0.0041	0.9516 ± 0.0052
	2	0.9538 ± 0.0077	0.9519 ± 0.0077
	10	0.9322 ± 0.0072	0.9415 ± 0.0062
	20	0.9125 ± 0.0131	0.9184 ± 0.0150

[a] Values are mean ± SD; n = 3. [b] Significantly different from control at $p < 0.05$. [c] Significance, $0.05 < p < 0.1$.

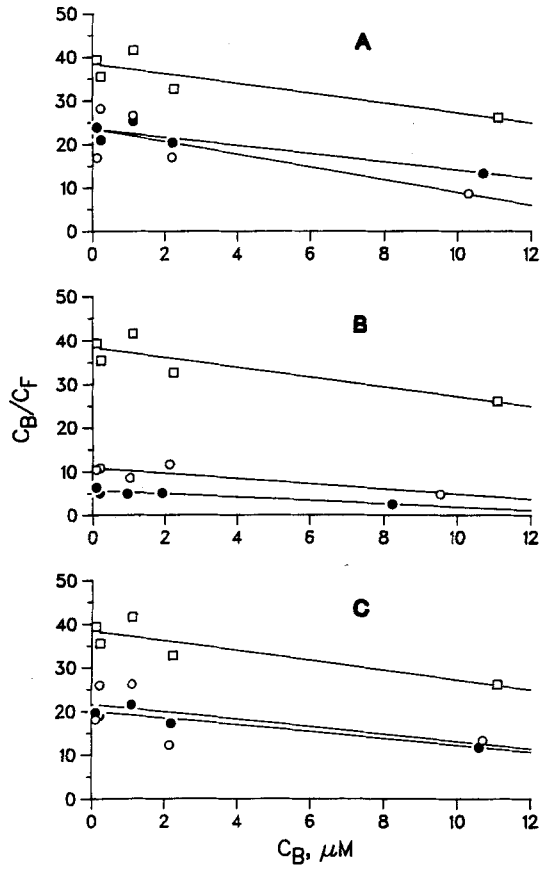

Figure 1—Scatchard plots illustrating the binding of [^{3}H]fluvastatin in diluted HSA solution (29 μM) with or without warfarin (**A**), salicylic acid (**B**), and glyburide (**C**). Key: (□) fluvastatin alone; the low (○) and high (●) concentrations of co-solute were, respectively, 1 and 10 μg/mL for warfarin, 50 and 150 μg/mL for salicylic acid, and 0.1 and 1 μg/mL for glyburide.

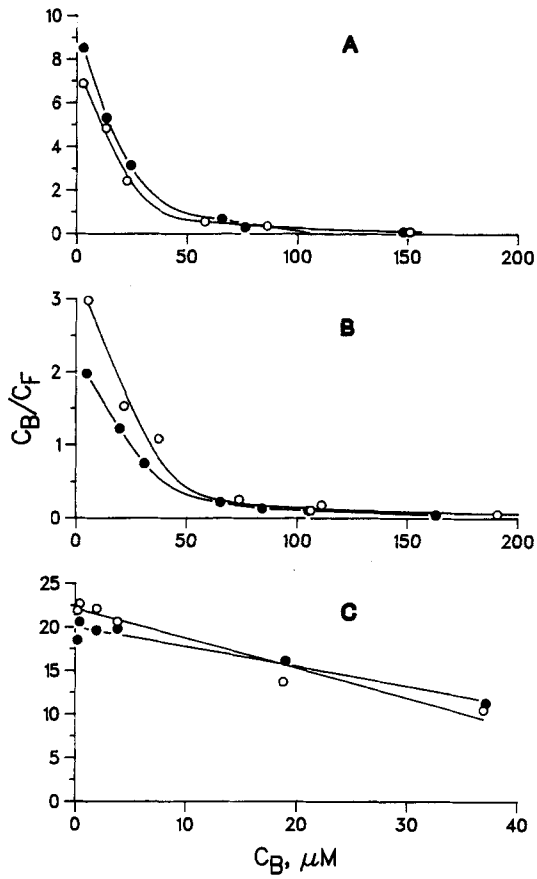

Figure 2—Scatchard plots illustrating the binding of [^{14}C]warfarin (**A**), [^{14}C]salicylic acid (**B**), and [^{14}C]glyburide (**C**) in diluted HSA solution (29 μM) with (●, 0.1 μg/mL) or without (○) fluvastatin.

was probably due to the low drug concentrations tested relative to that which saturates HSA binding capacity.

Conclusions

In the rat, dog, and human, fluvastatin was highly bound to plasma proteins over the wide range of concentrations tested. Binding appeared to be concentration dependent in the rat but not in the dog and human. Fluvastatin enantiomers 2 and 3 were >99% bound in normal human plasma, the binding of each being unaffected by the presence of the other. A major fluvastatin-binding protein in human plasma was albumin, whereas binding to α_1-acid glyburide was relatively weak and concentration dependent. Due to strong binding to plasma proteins, only a minor portion of the fluvastatin in blood was taken up by blood cells. Data obtained with human blood showed that the plasma:blood fluvastatin ratio was independent of temperature under the testing conditions.

At therapeutic concentrations in normal human plasma, the binding of fluvastatin to proteins was unaffected by

Table X—Binding Parameters to HSA (29 μM)

Drug	Co-Solute	Co-Solute Concentration, μg/mL	Primary Binding Site			Secondary Binding Site			Total
			n_1	k_1, $M^{-1} \times 10^{-5}$	K_1, $M^{-1} \times 10^{-5}$	n_2	k_2, $M^{-1} \times 10^{-3}$	K_2, $M^{-1} \times 10^{-3}$	$(K_1 + K_2)$, $M^{-1} \times 10^{-5}$
[^{3}H]Fluvastatin	—[a]		1.17	11.3	13.2	NA[b]	NA	NA	13.2
	Warfarin	1	0.55	14.7	8.09	NA	NA	NA	8.09
	Warfarin	10	0.85	9.49	8.07	NA	NA	NA	8.07
	Salicylic Acid	50	0.62	5.98	3.71	NA	NA	NA	3.71
	Salicylic Acid	150	0.51	3.87	1.97	NA	NA	NA	1.97
	Glyburide	0.1	0.87	8.55	7.44	NA	NA	NA	7.44
	Glyburide	1	0.88	7.88	6.93	NA	NA	NA	6.93
[^{14}C]Warfarin	—		1.03	2.45	2.52	5.84	2.01	11.7	2.64
	Fluvastatin	0.1	0.88	3.36	2.96	2.94	9.18	27.0	3.23
[^{14}C]Salicylic acid	—		1.46	0.77	1.12	10.1	0.35	3.55	1.16
	Fluvastatin	0.1	1.32	0.56	0.73	6.25	0.60	3.75	0.77
[^{14}C]Glyburide	—		2.24	3.41	7.64	NA	NA	NA	7.64
	Fluvastatin	0.1	3.04	2.27	6.90	NA	NA	NA	6.90

[a] —, No co-solute. [b] NA, Not applicable.

warfarin, salicylic acid, and glyburide. Similarly, fluvastatin had no influence on the binding of these compounds to plasma proteins. In diluted HSA solution (29 μM), bound fluvastatin was displaced by all three co-solutes tested, with salicylic acid showing the strongest effect. Conversely, fluvastatin reduced the binding of salicylic acid but had little effect on that of warfarin and glyburide, probably because of the relatively weak affinity of salicylic acid for albumin. It is noted that the albumin concentration used is ~22 times more dilute than normal serum levels and would be highly unlikely encountered in clinical practice.

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