Effect of a Major Metabolite on the Plasma Protein Binding of Tolmetin

Keyphrases □ Tolmetin—effect of a major metabolite on plasma protein binding □ Metabolites—effect on the plasma protein binding of tolmetin □ Plasma protein binding—effect of a major metabolite, tolmetin

To the Editor:

The nonsteroidal anti-inflammatory agent, tolmetin, is highly bound (>99%) to plasma proteins (1). Recently, Selley *et al.* (2) reported that 3 μg/ml (10.4 μ*M*) of 1-methyl-5-(4-carboxybenzoyl)-1*H*-pyrrole-2-acetic acid (I), a major metabolite of tolmetin (3), caused a marked (245%) increase in tolmetin free fraction when dialyzed until equilibrium against 4% human serum albumin containing 26.4 μg/ml (84 μ*M*) of tolmetin. Such a result is unusual since these investigators also observed that I was bound only 72% to human serum albumin. This implies that the metabolite may have less affinity for albumin binding sites than tolmetin; yet, small concentrations of I were capable of displacing the extensively bound parent drug. We decided to investigate further the effect of I on tolmetin binding.

Plasma was obtained from three healthy volunteers and was seeded with 0 (control sample), 0.8 (0.23 μg/ml), 8 (2.3 μg/ml), 80 (23 μg/ml), and 800 μ*M* (230 μg/ml) concentrations of I[1] and dialyzed against 0.125 *M* Sørenson's sodium–potassium phosphate buffer (pH 7.4) containing 80 μ*M* (25.2 μg/ml) of [^{14}C]tolmetin (14.8 μCi/mg)[1]. Equilibrium was achieved after 4 hr of dialysis using an equilibrium dialysis system[2] equipped with a microcell accessory and cellulose dialysis membranes (MW cutoff, 6000–8000)[3]. This experiment was also performed using human serum albumin[4] instead of human plasma. Aliquots (100 or 150 μl) of dialysate and dialyzed plasma were assayed for radioactivity in 10 ml of scintillation cocktail[5] using a liquid scintillation spectrometer[6] and external standard quench correction.

In a second series of experiments, plasma aliquots (0.2 ml each) from 10 healthy volunteers were dialyzed against Sørenson's buffer (0.2 ml) containing 0.5, 5, and 50 μg/ml of [^{14}C]tolmetin, and the tolmetin free fraction was determined at each concentration. In addition, samples (1.0 ml) of pooled human plasma were seeded with 2, 20, and 200 μg/ml of I and dialyzed against 1.0-ml aliquots of 0.067 *M* Sørenson's buffer. Buffer and plasma samples (0.5 ml) were assayed for I using a sensitive HPLC procedure[7]. The percentage of free (unbound) I or tolmetin was calculated using:

$$\frac{C_d}{C_p} \times 100 = \% \text{ free} \qquad \text{(Eq. 1)}$$

where C_d is the concentration of ligand in the dialysate (unbound) and C_p is the concentration of ligand in dialyzed plasma (bound and unbound).

Table I lists the results obtained when 80 μ*M* [^{14}C]tolmetin was dialyzed in the presence of increasing concentrations of I in both plasma and 4% human serum albumin. The metabolite produced no effect on tolmetin protein binding when added at equimolar concentrations or less. In contrast, tolmetin free fraction increased when I was added in 10-fold molar excess over tolmetin. These results are consistent with the observation that tolmetin was much more highly bound to plasma proteins than I over the broad range of concentrations studied (Table II). The metabolite may have less affinity for binding sites shared with tolmetin on plasma proteins (probably albumin). Under these conditions I would not be expected to displace any protein-bound tolmetin except when added in great excess. These data differ from the study by Selley *et al.* (2), who reported that addition of 3 μg/ml of I yielded an in-

Table II—Binding of Tolmetin and its Major Metabolite I to Human Plasma

Seeded Concentration, μg/ml[a]	Percent Free
Tolmetin	
0.5	0.37 ± 0.14[b]
5	0.37 ± 0.10
50	0.63 ± 0.11
I	
2	19.6 ± 0.7[c]
20	23.8 ± 1.2
200	26.5 ± 1.0

[a] Expressed as micrograms of free acid per milliliter. [b] Mean ± *SD* of binding to plasma from 10 healthy volunteers. [c] Mean ± *SD* of 3–5 aliquots of pooled human plasma dialyzed separately.

Table I—The Effect of Metabolite I on Tolmetin Binding to Human Plasma and to 4% Human Serum Albumin

Concentration of [^{14}C]Tolmetin		Concentration of I		Percent Free Tolmetin[a]	
μ*M*	μg/ml[b]	μ*M*	μg/ml[b]	Plasma	4% Human Serum Albumin (fatty acid free)
80	25.2	0	0	0.42 ± 0.03	0.52 ± 0.07
80	25.2	0.8	0.23	0.39 ± 0.01	0.57 ± 0.08
80	25.2	8	2.3	0.53 ± 0.08	0.62 ± 0.06
80	25.2	80	23	0.42 ± 0.05	0.57 ± 0.07
80	25.2	800	230	0.68 ± 0.18	0.92 ± 0.21

[a] Mean ± *SD* of 4 aliquots dialyzed separately. [b] Expressed as micrograms of free acid per milliliter.

[1] McNeil Pharmaceutical, Spring House, Pa.
[2] Dianorm, Diachema Ag., Rüschlikon, Switzerland.
[3] Spectra/Por, Spectrum Medical Industries, Inc., Los Angeles, Calif.
[4] Fatty acid free; Sigma Chemical Co., St. Louis, Mo.
[5] BioFluor, New England Nuclear, Boston, Mass.
[6] Searle Analytical 81, Searle Analytic Inc., Des Plaines, Ill.

[7] T. Snyderman, N. L. Renzi, Jr., and K. T. Ng, data on file, McNeil Pharmaceutical, Spring House, Pa.

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crease in free fraction of tolmetin (present at 26.7 μg/ml) from 0.29 ± 0.02% (Mean ± *SD*) to 1.0 ± 0.12%. It appears that factors other than the metabolite itself must have contributed to the increase in tolmetin free fraction observed by these investigators.

The fact that I does not readily displace tolmetin from plasma protein binding sites has important implications in the understanding of tolmetin disposition. Since the metabolite circulates at concentrations lower than those of tolmetin in healthy subjects (1, 4) and arthritic patients (5), no effect of I on tolmetin disposition would be anticipated based on the data presented in Table I. In anephric patients receiving tolmetin, I accumulates in plasma to concentrations often >10-fold those of tolmetin[8]. Some displacement of tolmetin by the metabolite in these patients might be expected. However, since uremic plasma has reduced binding affinity for tolmetin (6), it is difficult to assess the additional influence of high circulating metabolite concentrations on tolmetin disposition in the uremic patient.

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Hydroxyisolongifolaldehyde: A New Metabolite of (+)-Longifolene in Rabbits

Keyphrases □ Longifolene—hydroxyisolongifolaldehyde, a new metabolite in rabbits □ Metabolites—hydroxyisolongifolaldehyde from longifolene in rabbits □ Hydroxyisolongifolaldehyde—a new metabolite of longifolene in rabbits

To the Editor:

In spite of daily usage of terpenoid-containing plant products such as fruits, drinks, tobacco, *etc.*, the metabolic fate of terpenoids is essentially unknown. The aim of this study is to clarify terpenoid biotransformation in mammalian biochemistry from the toxicological point of view. This will provide guidance for the effective usage of natural products and for preparation of starting materials for organic synthesis.

Wild rabbits damage the forests in Japan by feeding on the artificially planted young *Chamaecyparis obtusa*, an important tree used commercially in Japan. This Japanese cypress contains longifolene (I) as a major sesquiterpene hydrocarbon. The metabolism of α-pinene, camphene, and other related terpenoids in rabbits was studied previously (1–7).

Longifolene (36 g), $[\alpha]_D$ = +39.3°, in chloroform, was administered to rabbits and the metabolites were isolated according to the method described previously. The neutral crude alcohols (3.7 g), were acetylated and purified to afford the main acetate (II) (17% for total acetates), which gave the following spectral data: mass spectrum: *m/z* 278 ($C_{17}H_{26}O_3$); 2700 and 1710 cm^{-1}; and a small doublet (J = 1.5 Hz) at 9.88 ppm of an aldehyde group. It was hydrolyzed by potassium carbonate in methanol and on silica gel chromatography gave pure metabolic alcohol (III). The parent ion of III, *m/z* 236, means a molecular formula $C_{15}H_{24}O_2$ showing that two oxygen atoms were introduced into longifolene. These oxygen atoms are based on a hydroxyl group (3450 cm^{-1}) and an aldehyde group (2700 and 1720 cm^{-1}; 9.87 ppm). The signals at 3.46 and 3.31 ppm (AB quartet) of this alcohol shifted 0.5 ppm upfield compared with those of acetate, suggesting the presence of a primary hydroxyl group. The olefin group of longifolene was lost in this alcohol. Thus, the change of this *exo*-methylene group to an aldehyde or primary alcohol was expected. The positions of newly introduced groups were determined on the basis of IR, ^{1}H-NMR, and ^{13}C-NMR spectra.

First, the aldehyde proton signal of III split into a doublet (J = 2.0 Hz) indicating the presence of an adjacent 7-H proton. By this splitting, it was concluded that the position of the aldehyde group is not at C-12, C-14, and C-15 but at C-7. The 7-H configuration was determined on the basis of a coupling constant. Provided that 7-H is in an α-configuration, the coupling constants of this proton would be anticipated to be $J_{7,1}$ = 1.3–2.6 Hz and $J_{7,8}$ = ~0.5 Hz. However, if 7-H is in a β-configuration, these values would be expected to be: $J_{7,8}$ = 3.6–5.0 Hz, $J_{7,9}$ ~1.2 Hz, and $J_{7,11}$ = 0.5 Hz. In fact, $J_{7,8}$ in III was observed as 3.5 Hz meaning 7-H has the β-configuration. Thus, the configuration of this aldehyde group was determined to be in the C-7-*endo*-form, which is stable in isolongifolaldehyde (8).

Second, in the IR spectra of the metabolized alcohol and

Table I—^{13}C-NMR Chemical Shifts in Longifolene and Hydroxyisolongifolaldehyde

	Longifolene (I)	Hydroxyisolongifolaldehyde (III)
C-1	62.2	56.3
C-2	33.6	37.8
C-3	36.5	34.2
C-4	21.2	21.0
C-5	43.4	44.2
C-6	44.1	43.0
C-7	168.0	59.9
C-8	48.0	40.8
C-9	29.8	25.8
C-10	25.5	23.0
C-11	45.2	47.0
C-12	30.1[a]	26.0[a]
C-13	99.0	206.4
C-14	30.6[a]	22.6[a]
C-15	30.6[a]	72.6

[a] Assignments may be reversed in each compound.

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