Effect of Pirprofen on Protein Binding of Warfarin and Tolbutamide in Human Plasma

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Received January 21, 1981, from the *Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Ardsley, NY 10502.* Accepted for publication May 14, 1981.

Abstract □ The extent of warfarin and tolbutamide binding to plasma proteins was determined with and without pirprofen by an ultrafiltration procedure employing ^{14}C-labeled drugs. Results from *in vitro* studies at 37° showed that the degree of binding amounted to 97.8% for warfarin and 95.6% for tolbutamide. The binding characteristics of these drugs were not altered when plasma containing either warfarin or tolbutamide at concentrations equivalent to those expected normally after therapeutic dosing were concomitantly spiked with therapeutic amounts of pirprofen. Consequently, potentiation resulting from drug displacement would not be anticipated in humans when pirprofen is administered along with warfarin or tolbutamide.

Keyphrases □ Pirprofen—effect on protein binding of warfarin and tolbutamide □ Tolbutamide—protein binding in presence of pirprofen □ Warfarin—protein binding in presence of pirprofen □ Binding, protein—tolbutamide and warfarin

Pirprofen, 3-chloro-4-(2,5-dihydro-1*H*-pyrol-1-yl)-α-methyl benzeneacetic acid, is a new nonsteroidal anti-inflammatory agent which is well tolerated and highly effective in the treatment of rheumatoid arthritis (1–3). Investigations in these laboratories showed that pirprofen is bound to plasma proteins to an extent greater than 99.8%[1]. Concurrent salicylate administration causes a reduction of plasma pirprofen concentrations of ~31% in rats (4) and ~38% in humans[1].

BACKGROUND

It has been well established that concomitant administration of two highly protein-bound drugs can lead to a displacement of one drug by the other when the two drugs compete for the same binding sites (5, 6). Drug displacement results in the availability of more free or unbound drug, which, in turn, might lead to a change in the distribution and elimination of the affected drug (7, 8). Certain nonsteroidal anti-inflammatory drugs, such as phenylbutazone, when administered concomitantly with warfarin cause total plasma warfarin concentrations to decrease by 50% while free drug increases to 65% (9). A study in rats showed that ibuprofen increased total clearance and the anticoagulant effect of warfarin (10); however, human serum containing ibuprofen showed only a 10% increase in free warfarin (11). Naproxen caused a partial displacement (14–17%) of warfarin (12).

Since arthritics undergoing chronic pirprofen therapy also might receive other drugs concomitantly, it was important to study the relationship between pirprofen concentrations and protein binding of drugs likely to be administered with pirprofen. The results of *in vitro* binding studies with warfarin and tolbutamide are discussed in this paper.

EXPERIMENTAL

Pooled heparinized human plasma was obtained from healthy volunteers and spiked with either [^{14}C]warfarin[2] (1 or 10 μg/ml) or [^{14}C]tolbutamide[3] (20, 40, 100, or 200 μg/ml) in the presence of pirprofen[4] (0, 25, 50, 100, or 200 μg/ml).

The spiked samples were allowed to equilibrate at room temperature for at least 1 hr. Plasma aliquots, 1 or 2 ml, of each sample were pipetted into membrane cones[5], which had been soaked previously in distilled water for a minimum of 1 hr. These membranes were contained in conical supports[6], which were placed inside centrifugation tubes[7]. To ensure a uniform filtration, the seam of the membrane cone was faced toward the rotating direction. A prerun centrifugation[8] of 12 minutes (~1000×*g*) was necessary to eliminate the water content of the membrane. The ultrafiltrate (~300 μl) was collected after a second centrifugation (7 min) at the same speed. Another group of samples was carried through the same procedure (equilibration and treatment of filter cones) at 37 ± 2°. The centrifugation at elevated temperature was accomplished by placing the centrifuge inside a large oven maintained at 37 ± 2°.

Aliquots of 10–150 μl of the spiked plasma samples and of the ultrafiltrate were mixed with 15 ml of scintillation cocktail[9] and assayed for carbon 14 by liquid scintillation spectrometry[10]. Unbound warfarin and tolbutamide were expressed in percent and were obtained from the ratio of radioactivity measurements in the ultrafiltrate (unbound drug) to those in whole plasma (total drug). Means and standard deviations were obtained from four analyses.

Recoveries of warfarin and tolbutamide from the filtration cones were determined prior to the binding studies. Two milliliters of a pH 7.4 buffer solution containing 199.8 ng of tolbutamide/ml or 10.5 ng of warfarin/ml was added to the filtration cones and carried through the described procedure. Aliquots of 400 μl of the filtrate were then taken for radioactivity measurements. The data obtained from four replicate analyses (Table I) gave average recoveries of 99 ± 0.4% for tolbutamide and 92 ± 0.5% for warfarin.

Table I—Percent Recovery of [^{14}C]Tolbutamide and [^{14}C]-Warfarin from Filtration Cones

Drug	Concentration, ng/ml	Determinations[a] 1	2	3	4	Average ±*SD*
Warfarin	10.5[b]	92	92	91	92	92 ± 0.5
Tolbutamide	199.8[c]	98	99	99	99	99 ± 0.4

[a] Room temperature (22 ± 2°). [b] Equivalent to the amount of free drug in a plasma sample containing 1 μg of warfarin/ml. [c] Equivalent to the amount of free drug in a plasma sample containing 4 μg of tolbutamide/ml.

RESULTS AND DISCUSSION

Warfarin—The results of binding studies at 37° (Fig. 1) indicate that the amount of free or unbound warfarin was 2.2 ± 0.15% (mean ± *SD*) for drug levels of 1–10 μg/ml. Conversely, 97.8% of the drug was bound to plasma protein. The data obtained at room temperature indicate about one-half as much unbound warfarin (1.1%). Yacobi and Levy (12) reported that 99.1% of the drug was bound to serum proteins for serum drug concentrations of 1.25 μg/ml.

When the warfarin samples (37°) were spiked with 50 μg/ml of pirprofen, a mean of 2.4 ± 0.24% (*SD*) was found; at 100 μg/ml, free warfarin averaged 2.3 ± 0.06% (*SD*). For these concentrations, pirprofen did not significantly ($p = 0.05$) alter warfarin binding. Since peak plasma concentrations of 23–44 μg/ml occur after chronic dosing with pirprofen (13) and since warfarin concentrations at steady state range from 1.6 to 4.1 μg/ml (9, 14), one would not expect warfarin to be displaced when therapeutic amounts of pirprofen are administered concomitantly with the anticoagulant. However, at pirprofen concentrations of 200 μg/ml or approximately four times the maximum levels found clinically, free

[1] To be published.
[2] Specific activity of 49 mCi/mmole, Amersham, Chicago, Ill.
[3] Specific activity of 47.6 mCi/mmole, New England Nuclear, Boston, Mass.
[4] Ciba-Geigy Corp., Ardsley, N.Y.
[5] CF-50, Amicon Corp., Lexington, Mass.
[6] CSIA, Amicon Corp., Lexington, Mass.
[7] CTI, Amicon Corp., Lexington, Mass.
[8] CT 1300, Adams Dynac, Raycomm Industries, Inc., Freehold, N.J.
[9] Scintisol, Isolab, Inc., Akron, Ohio.
[10] SL 4000, Intertechnique, Fairfield, N.J.

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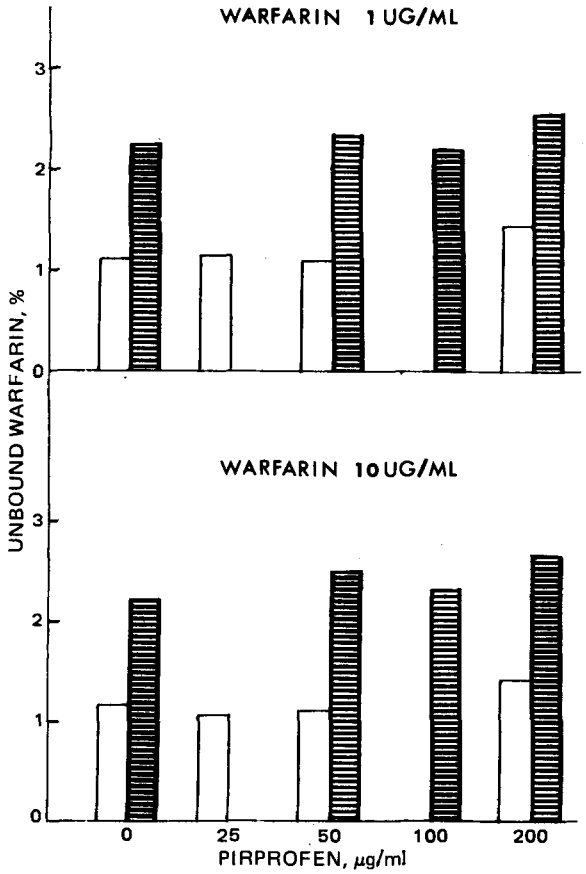

Figure 1—*Effect of pirprofen on the* in vitro *plasma binding of warfarin. Key:* □, *room temperature; and* ▤, *37°.*

warfarin increased by 27 (room temperature) and 20% (37°).

Tolbutamide—The extent of binding to plasma proteins was 95.6 ± 0.15% (mean ± *SD*) at 37° for plasma tolbutamide levels between 40 and 100 µg/ml (Fig. 2). Similar results were obtained at room temperature for tolbutamide concentrations between 20 and 100 µg/ml. These values are comparable to those of Miller *et al.* (15), who reported 96.8 and 96.0% binding in young and old subjects, respectively, for plasma drug concentrations of 100 µg/ml, and to those of Zilly *et al.* (16), who found 95% binding following a 20-min intravenous infusion of 1600 mg of tolbutamide.

At 37°, no change in tolbutamide binding (95.6 ± 0.17%; $p = 0.05$) was observed when plasma samples containing 40–100 µg of tolbutamide/ml were spiked with pirprofen at levels up to 50 µg/ml. An increase in free tolbutamide was noted, however, for higher tolbutamide concentrations. As observed in Fig. 2, free tolbutamide increased to 8.0 (room temperature) and 9.9% (37°) for tolbutamide levels of 200 µg/ml. Although levels of this magnitude are not normally maintained after dosing with tolbutamide (17–19), the *in vitro* binding data suggest that binding sites were nearing saturation, resulting in greater availability of unbound drug. At these high concentrations, there is a suggestion that pirprofen can partially displace tolbutamide. The results (room temperature and 37°) show that the addition of 50 µg of pirprofen/ml increased free tolbutamide to ~11% of the total drug fraction.

In conclusion, pirprofen did not alter the binding characteristics of tolbutamide or warfarin when these compounds were added to plasma (*in vitro*) at levels expected normally after therapeutic dosing. Consequently, a change in pharmacological response from warfarin or tolbutamide resulting from drug displacement would not be anticipated in humans when either of these compounds is administered concomitantly with pirprofen.

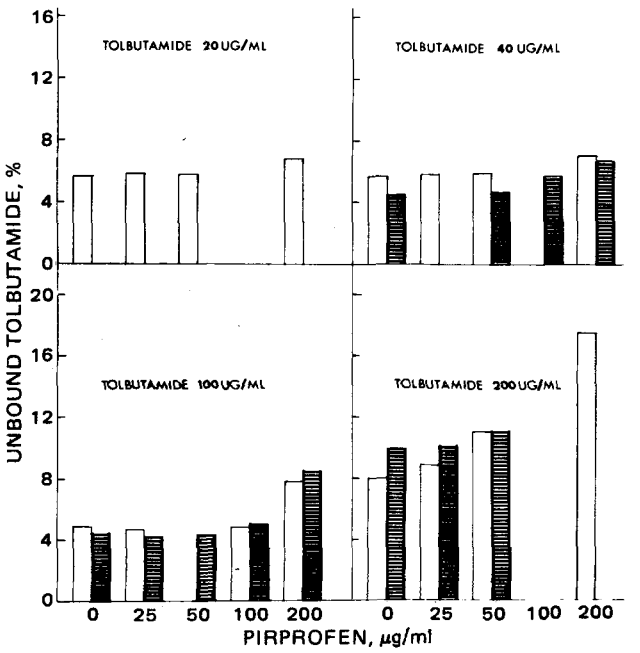

Figure 2—*Effect of pirprofen on the* in vitro *plasma binding of tolbutamide. Key:* □, *room temperature; and* ▤, *37°.*

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