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Plasma Protein Binding of Zomepirac Sodium

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Abstract □ The plasma protein binding of zomepirac, a new nonnarcotic analgesic, was studied using equilibrium dialysis. Experiments were performed using human plasma and plasma from mice, rats, and rhesus monkeys, all species of pharmacological or toxicological interest. At concentrations approximating those achieved *in vivo*, the binding was fairly constant at 98–99% in all species except the rhesus monkey, where binding was decreased from 98 to ~96% at higher concentrations (>50 μg/ml). Zomepirac (10 μg/ml) did not appear to displace or to be displaced by warfarin (10 μg/ml) in human plasma. However, salicylate (5–200 μg/ml) caused a concentration-dependent decrease in zomepirac (10 μg/ml) binding. Zomepirac did not affect salicylate binding.

Keyphrases □ Zomepirac sodium—plasma protein binding studies in plasma from humans, rats, mice, and rhesus monkeys, interaction studies with warfarin and salicylic acid □ Plasma protein binding—zomepirac sodium in plasma from humans, rats, mice, and rhesus monkeys □ Warfarin—interaction studies with zomepirac sodium, effect on plasma protein binding □ Salicylic acid—interaction studies with zomepirac sodium, effect on plasma protein binding □ Analgesics, nonnarcotic—zomepirac sodium, plasma protein binding studies in humans, rats, mice, and rhesus monkeys, interaction studies with warfarin and salicylic acid

Plasma protein binding of drugs is an important factor in drug disposition (1–3). This report describes the interaction of zomepirac sodium, a new nonnarcotic analgesic agent (4–6), with plasma proteins from several species. Some preliminary binding interaction studies in human plasma are also reported.

EXPERIMENTAL

Materials—Zomepirac sodium [sodium 5-(4-chlorobenzoyl)-1,4-dimethyl-1*H*-pyrrole-2-acetate dihydrate] was labeled with carbon 14 at a specific activity of 10.18 μCi/mg[1]. The drug was 96–98% radiochemically pure by TLC at the time of use. [^{14}C]Salicylic acid[2] and [^{14}C]warfarin[2] were used at specific activities of 421 and 164 μCi/mg, respectively. Both drugs were 99% radiochemically pure by TLC.

Heparinized plasma was harvested from blood collected from Wistar rats[3], CD-1 Swiss mice[3], and rhesus monkeys[4]. Citrated human plasma was purchased locally. Sorensen's buffer (0.067 *M* phosphate, pH 7.4) was prepared by dissolving 1.72 g of monobasic potassium phosphate and 7.70 g of dibasic sodium phosphate in 1 liter of distilled water.

Methods—Binding experiments were performed on an equilibrium dialysis system[5]. Regenerated cellulose dialysis membranes (mol. wt. cutoff of 5000) were prepared by three rinses in distilled water and three rinses in buffer. The membranes were placed between two-piece polytef dialysis cells (1-ml volume per side) mounted in a spring-loaded rack, and each half-cell was filled. One side of the cell was filled with plasma, and the other side was filled with the drug in buffer.

The cells were rotated at 12 rpm for 2 hr at room temperature, and then the half-cells were emptied and assayed for total carbon 14 as a measure of zomepirac. These dialysis conditions were selected since preliminary experiments in the absence of plasma (*i.e.*, zomepirac dialyzed against buffer) showed that equilibrium was reached in 2 hr and that plasma binding was not different at room temperature or 37°. Furthermore, TLC analysis of selected samples showed that no degradation of zomepirac occurred under these conditions.

Interaction Studies—[^{14}C]Zomepirac was dialyzed against undiluted rat, mouse, monkey, and human plasma at concentrations ranging from 0.1 to 250 μg/ml, depending on the species. These concentrations were in the ranges of those observed after pharmacological doses of zomepirac sodium (7–10).

To assess the potential for displacement of zomepirac by other agents, experiments were performed with warfarin[6] and salicylic acid[7] using human plasma. The [^{14}C]zomepirac concentration was 10 μg/ml. Warfarin was tested at 10 μg/ml, and salicylic acid was tested from 5 to 200 μg/ml. When the effect of zomepirac on the binding of these agents was evaluated, a tracer of [^{14}C]warfarin (~10,000 dpm/ml, 0.03 μg/ml) or salicylate (~10,000 dpm/ml, 0.01 μg/ml) was added, and nonradioactive zomepirac was used.

Sample Analysis—Total radioactivity was determined by adding aliquots of both plasma and buffer solutions (after dialysis) to 10 ml of scintillation cocktail[8]. Samples were counted in a refrigerated liquid scintillation spectrometer and were corrected for quenching using the external standard method.

Selected samples were analyzed for drug decomposition during dialysis. Zomepirac was assayed by applying aliquots of the postdialysis plasma and buffer samples to silica gel TLC plates[9] developed in chloroform–methanol–acetic acid (94:5:1 v/v/v). Analysis was by either segmentation and liquid scintillation counting or radioscan. No decomposition was observed.

[1] McNeil Pharmaceutical, Spring House, Pa.
[2] Amersham Corp., Des Plaines, Ill.
[3] Charles River, Wilmington, Mass.
[4] Primate Imports, Port Washington, N.Y.
[5] Dianorm, Diachema Ag., Ruschlikon, Switzerland.
[6] Endo Laboratories, Garden City, N.Y.
[7] J. T. Baker Chemical Co., Phillipsburg, N.J.
[8] Biofluor, New England Nuclear, Boston, Mass.
[9] GF 254 (250 μm), Analtech, Newark, Del.

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Table I—Zomepirac Binding to Plasma Proteins of Various Species

Species	Zomepirac Concentration[a], μg/ml								
	250	125	50	20	10	5	1	0.5	0.1
Human	—[b]	—	—	—	98.6 ± 0.3	98.6 ± 0.03	98.9 ± 0.1	98.4 ± 0.1	98.5 ± 0.2
Rat	—	—	—	97.7 ± 0.1	97.9 ± 0.1	98.9 ± 0.4	98.0 ± 0.3	98.1 ± 0.3	98.6 ± 0.5
Mouse	—	—	—	—	98.5 ± 0.6	98.1 ± 0.6	98.0 ± 0.2	99.1 ± 0.1	97.4 ± 0.4
Monkey	96.3 ± 0.4	96.4 ± 0.2	97.1 ± 0.4	98.4 ± 0.2	98.8 ± 0.5	98.9 ± 0.1	98.4 ± 0.1	99.1 ± 0.2	97.6 ± 0.8

[a] Values represent the starting concentration. Values represent mean percent bound ± *SD* of four to 10 determinations. [b] Not tested.

Table II—Protein Binding Interactions of Zomepirac with Warfarin and Salicylate

Zomepirac		Warfarin	
Concentration, μg/ml	Percent Bound[a]	Concentration, μg/ml	Percent Bound[a]
0	—	10	98.3 ± 0.6
10	98.3 ± 0.1	10	98.5 ± 0.8
Zomepirac		**Salicylate**	
Concentration, μg/ml	Percent Bound[a]	Concentration, μg/ml	Percent Bound[a]
0	—	5	94.5 ± 0.2
0	—	25	93.7 ± 0.3
0	—	100	85.4 ± 0.6
10	98.1 ± 0.3	5	94.5 ± 0.4
10	97.3 ± 0.3	25	93.1 ± 0.4
10	95.9 ± 0.7	100	83.7 ± 0.5
10	92.8 ± 0.6	200	—

[a] Mean ± *SD* of five determinations.

Salicylic acid was assayed by pooling plasma or buffer samples (~2 ml), adjusting to pH 1–2 with 0.5 ml of 6 *N* H_2SO_4, and extracting with 10 ml of ether. After shaking for 15 min and centrifuging for 5 min, an aliquot of the ether layer was counted for carbon 14 and the remainder was evaporated to dryness under nitrogen. More than 95% of the radioactivity was extracted. The residue was dissolved in a small amount of methanol and spotted on a TLC plate. The plate was developed in toluene–dioxane–acetic acid (90:24:4 v/v/v) and analyzed as described earlier. No salicylic acid degradation was observed.

Warfarin samples were pooled as described for salicylate and extracted with 10 ml of ethylene dichloride (15 min of shaking and 10 min of centrifugation). An aliquot of the organic layer was counted for carbon 14, and the remainder was evaporated to near dryness. Approximately 80% of the radioactivity was extracted. The extract was spotted on a TLC plate, and the plate was developed in toluene–ethyl formate–formic acid (5:4:1 v/v/v). Only 59% of the radioactivity in the dialysate corresponded to [^{14}C]warfarin, while essentially all of the carbon 14 in the plasma was unchanged warfarin. Therefore, the buffer was corrected for the presence of degradation products prior to calculating the extent of binding.

Percent bound values were calculated as follows:

$$\text{percent bound} = \frac{C_{b+f} - C_f}{C_{b+f}} \times 100 \qquad \text{(Eq. 1)}$$

where C_{b+f} is the drug concentration on the plasma side of the membrane (bound plus free drug) and C_f is the drug concentration on the buffer side (free drug).

RESULTS AND DISCUSSION

Results of experiments evaluating the relationship between zomepirac concentration and extent of binding are shown in Table I. The mean percent bound to human plasma was essentially constant from 0.1 to 10 μg/ml, ranging from 98.4 to 98.9%. Similar findings were observed in the other species, although binding to monkey plasma was reduced slightly with higher zomepirac concentrations (≥50 μg/ml).

The results of the binding interaction studies in human plasma are shown in Table II. Warfarin (10 μg/ml) had no effect on zomepirac (10 μg/ml) binding, even though both drugs were tested at high concentrations relative to the those achieved after therapeutic doses of the drug (10, 11). Similarly, zomepirac did not alter warfarin binding. The effect of salicylic acid on zomepirac plasma binding is shown in Table II. At all concentrations tested, salicylate caused a statistically significant reduction in zomepirac binding, thus resulting in higher free drug concentrations. At 200 μg of salicylate/ml, this effect would result in a change in free fraction from 0.014 to 0.072, a fivefold increase. Zomepirac had no effect on salicylate binding.

Thus, similar to other acidic compounds (12, 13), zomepirac was highly bound to plasma proteins. Based on these preliminary studies, *in vivo* interactions of zomepirac and warfarin resulting from protein binding effects apparently are unlikely. While the potential for interactions with salicylate (*i.e.*, aspirin) exists, the *in vivo* importance cannot be predicted (14). Furthermore, these studies do not rule out drug interactions due to metabolic effects, additive pharmacological effects, or effects of drug metabolites.

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