Radioimmunoassay for Bupropion in Human Plasma: Comparison of Tritiated and Iodinated Radioligands

Robert F. Butz, Phillip G. Smith, David H. Schroeder, and John W. A. Findlay

We evaluated the potential usefulness of $^{125}$I-labeled $p$-hydroxybupropion in a direct radioimmunoassay for bupropion in human plasma as compared with a currently used $[^3]$H]bupropion dextran-coated charcoal method. In both radioimmunoassay methods succinoypropylbupropion antiserum was used that was highly specific for unchanged drug. Cross reactivities with known bupropion metabolites being less than 0.3%. However, the use of $^{125}$I-labeled $p$-hydroxybupropion afforded greater sensitivity (0.3 μg/L vs 0.6 μg/L with $[^3]$H]bupropion) and was readily adaptable to the more convenient polyethylene glycol separation method. Between-assay CVs were 3.8 to 12.2% (mean 7.6%) with the $^{125}$I-based radioimmunoassay and 5.1 to 11.5% (mean 7.5%) with the $[^3]$H-based assay. Agreement between the two radioimmunoassay determinations of bupropion in human plasma samples collected over a 60-h period after oral drug administration was excellent (slope = 1.068, $r = 0.989$). We find the $^{125}$I-based assay a convenient and suitable alternative to the $[^3]$H]bupropion assay in pharmacokinetic studies in humans.

Additional Keyphrases: pharmacokinetics • drug assay

Bupropion hydrochloride (dl-2-tert-butylamino-3'-chloropropiophenone hydrochloride; Wellbutrin®; Table 1, I) is a novel antidepressant compound relatively free of the troublesome side effects frequently associated with monoamine oxidase inhibitors and tricyclic antidepressants (1). Multi-center placebo-controlled studies (2) have demonstrated the clinical efficacy of bupropion in relieving depression, and the drug is currently being evaluated at numerous medical centers in the U.S. and Europe. Concentrations of bupropion in plasma have been determined by "high-pressure" liquid chromatography (3) and by a radioimmunoassay procedure involving $[^3]$H]bupropion and a dextran-coated charcoal separation method (4). The radioimmunoassay has been used to investigate the pharmacokinetics of bupropion in healthy volunteers (5). However, the use of $[^3]$H]bupropion requires scintillation counting, which is expensive and restricts these assays to laboratories that are appropriately equipped.

We have developed recently a radioiodinated ligand, tentatively named $^{125}$I-labeled $p$-hydroxybupropion (II), for the bupropion radioimmunoassay, which we have used to measure bupropion concentrations in rodent plasma and brain (6). However, the presence in these samples of cross-reacting metabolites, including a compound tentatively identified as $p$-hydroxybupropion, necessitated solvent extraction from an alkaline medium before radioimmunoassay. We also found that human urine contains sufficient concentrations of unidentified cross-reacting metabolites to necessitate extraction of bupropion before radioimmunoassay by the $[^3]$H]bupropion dextran-coated charcoal method (5), whereas the direct analysis of human plasma samples showed only slight interferences (4).

Here we describe the development of an $^{125}$I-labeled $p$-hydroxybupropion-based radioimmunoassay in which polyethylene glycol precipitation is used to separate antibody-bound from free radioligand, and report the application of this procedure to direct human plasma analyses. We compared results obtained by both radioimmunoassay methods for 89 plasma samples collected from five subjects over a 60-h period after they ingested 100 mg of bupropion hydrochloride. We found that the method involving $^{125}$I-labeled $p$-hydroxybupropion and polyethylene glycol afforded greater convenience and assay sensitivity without sacrificing specificity or precision.

Materials and Methods

Materials

$[^6]$H]Bupropion hydrochloride (specific activity, 20.5 kCi/mol) was prepared by Drs. J. A. Hill and J. D. Scharver, Wellcome Research Laboratories, Research Triangle Park, NC. 2-(tert-Butylamino)-3'-125I]-4'-hydroxypropiophenone ($^{125}$I-labeled $p$-hydroxybupropion) was prepared as de-
scribed previously (5). Bupropion hydrochloride and 50-mg bupropion hydrochloride tablets were supplied by Burroughs Wellcome Co., Greenville, NC. dl-(erythro)-2-(tert-butylamino)-1-(3-chlorophenyl)propanol hydrochloride (III), dl-(threo)-2-(tert-butylamino)-1-(3-chlorophenyl)propanol hydrochloride (IV), dl-2-(tert-hydroxybutylamino)-3'-chloro-4'-hydroxypropionophenone (V), and dl-(erythro, threo)-2-(tert-hydroxybutylamino)-1-(3-chlorophenyl)propanol hydrochloride (VI) were provided by Dr. N. B. Mehta, Welcome Research Laboratories. dl-2-(tert-Butylamino)-3'-chloro-4'-hydroxypropionophenone (p-hydroxybupropion; VII) was prepared as described previously (5). Bupropion antiserum was elicited in rabbits immunized with a succinylpropylbuproprion-bovine serum albumin conjugate, as reported previously (5).

Assay buffer (pH 7.0) for the [3H]bupropion radioimmunoassay contained, per liter, 50 mmol of Na2HPO4/NaH2PO4, 150 mmol of NaCl, 10 mmol of Na2EDTA, and 1 g of gelatin (G-PBS). Assay buffer for the 125I-labeled p-hydroxybupropion radioimmunoassay was pH 7.4 and contained, per liter, 50 mmol of Na2HPO4/NaH2PO4, 150 mmol of NaCl, 10 mmol of Na2EDTA, and 0.2 g of bovine serum albumin (BSA-PBS). We separated antibody-bound and free [3H]bupropion by use of a suspension of charcoal (radioimmunoassay grade; Becton Dickinson & Co., Orangeburg, NY 10962; 5 g/L) in G-PBS also containing 2.5 g of dextran T-70 (Pharmacia Fine Chemicals, Piscataway, NJ 08854) per liter. The dextran-coated charcoal suspension was equilibrated for 30 min at 0 °C before use. We separated antibody-bound and free 125I-labeled p-hydroxybupropion by use of a solution which contained, per liter, 350 g of polyethylene glycol 8000, 50 mmol of Na2HPO4/NaH2PO4, 150 mmol of NaCl, and 10 mmol of Na2EDTA, at pH 7.4.

All data are the means of duplicate determinations.

Radioimmunoassay Procedures

Antiserum titrations. Antiserum titrations with [3H]bupropion were determined by mixing, in polystyrene tubes, 0.7 mL of various dilutions of antiserum in G-PBS with 0.2 mL of radioligand (0.45 ng of the base, containing approximately 10 000 cpm) in G-PBS and 0.1 mL of blank human plasma. Total-count and background tubes contained [3H]bupropion, blank human plasma, and G-PBS. After incubating samples overnight at 4 °C, we added 0.5 mL of a well-stirred, ice-cold suspension of dextran-coated charcoal to all except the total-count tubes. The tube contents were vortex-mixed, incubated at 4 °C for 5 min, and centrifuged (3000 × g) for 15 min at 4 °C. Supernates were decanted into 10 mL of scintillation cocktail and antibody-bound radioactivity was quantified with a Model 2600 scintillation spectrometer (Packard Instrument Co., Downers Grove, IL 60515). The titer of the antiserum used in these studies with [3H]bupropion (i.e., the antiserum dilution that bound specifically 40% of the total radioligand added) was 1:5000.

Antiserum titrations with 125I-labeled p-hydroxybupropion were performed by incubating, in glass tubes, 0.7 mL of antiserum dilutions in BSA-PBS with 0.2 mL of radioligand (80 000 cpm) in BSA-PBS and 0.1 mL of blank human plasma as described above. The total-count tubes contained only 125I-labeled p-hydroxybupropion; the background tubes contained labeled p-hydroxybupropion, blank human plasma, and BSA-PBS. We added 1.0 mL of polyethylene glycol solution to all except the total-count tubes, vortex-mixed the tube contents thoroughly, and centrifuged the tubes at ≥1000 × g and 4 °C for 30 min. Supernates were decanted, the inverted tubes were allowed to drain for 30 min, and the antibody-bound radioactivity in the pellets was quantified within a Model 5260 Auto-Gamma scintillation spectrometer (Packard Instrument Co.). The titer of the antiserum used in these studies with 125I-labeled p-hydroxybupropion (i.e., the dilution that produced 50% of maximum specific radioligand binding) was 1:12 000.

Antiserum specificity. The procedures were similar to those described for antiserum titrations, except that the blank human plasma was replaced by increasing concentrations of bupropion and compounds of interest in 0.1 mL of human plasma, and antiserum were used at the determined titers. Standard curves for each compound were expressed as the percentage B/B0 vs log drug concentration, in which B0 represents the background corrected amount (counts) of radioligand bound in the absence of unlabeled bupropion and B is the amount bound at a given drug concentration. Cross reactivities were expressed as the percentage ratio of the bupropion concentration required to produce 50% inhibition of radioligand binding to antiserum (IC50) to that of each compound of interest (7).

Analysis of unknown samples. We prepared a series of bupropion standard solutions—0.19 to 25 μg/L for the 125I radioimmunoassay and 0.39 to 50 μg/L for the 3H assay—in 0.1 mL of blank human plasma, and incubated them with the respective radioligands and appropriately diluted antiserum as described above. The total-count and background tubes for each assay method also were as described above. Unknown samples, or control samples containing known amounts of bupropion in human plasma (0.1 mL), diluted appropriately with blank plasma to enter the range of the assay, also were incubated with radioligand and antiserum. We assayed most controls and unknown samples at two dilutions. After overnight incubation, antibody-bound and free 125I-labeled p-hydroxybupropion and [3H]bupropion fractions were separated, and the standard curves were prepared as described above.

Human Study

After giving informed consent, five healthy men fasted overnight before and for 6 h after ingesting, with 200 mL of water, two 50-mg tablets of bupropion hydrochloride. Blood samples were collected into EDTA-containing Vacutainer Tubes (Becton Dickinson & Co., Rutherford, NJ 07070) by venipuncture at numerous times during the 60-h period after drug administration. Plasma samples were collected immediately and stored at −20 °C until analyzed by both of the radioimmunoassay methods. We used orthogonal regression analysis (8) to compare bupropion plasma concentration data determined by the two methods, and calculated the areas under the bupropion plasma concentration–time curves (AUC) by the trapezoidal rule method (9).

Results

Assay Characteristics

Specificity. Relative specificities of the [3H]bupropion and 125I-labeled p-hydroxybupropion radioimmunoassays are summarized in Table 1. Clearly, both radioimmunoassay methods were highly specific for bupropion, with known bupropion metabolites (compounds III–VI, m-chlorobenzoic acid, and m-chlorohippuric acid) (10, 11) cross reacting only very weakly (≤0.3%). Only p-hydroxybupropion (VII), which has not been identified as a bupropion metabolite in human plasma, cross reacted significantly with the succinylpropylbupropion antiserum used in these studies. Cross reactivities with various drugs, including catecholamines, narcotic analgesics, and tricyclic antidepressants, were <0.001% when tested with [3H]bupropion (4). Confirmation of the specificity of the [3H]bupropion radioimmunoassay was provided by the good agreement (slope = 1.15, r =
between bupropion concentrations in human plasma samples determined by radioimmunoassay and by a "high-pressure" liquid chromatographic method as reported recently (4). The specificity of the 

Sensitivity. Bupropion standard curves generated by both radioimmunoassay methods are shown in Figure 1. The limit of assay sensitivity, defined as the amount of unlabeled bupropion producing 10% inhibition of zero-dose radioligand binding, was 30 pg (corresponding to a concentration of 0.3 μg/L in a 0.1-mL sample) with the 

Values labeled

Comparative Analyses of Bupropion in Human Plasma

Figure 2 illustrates the good agreement between bupropion concentrations in human plasma samples determined by the [3H]bupropion and 125I-labeled p-hydroxybupropion radioimmunoassays. Curves for bupropion plasma concentration vs time, generated by analyses of plasma samples collected from one subject after ingestion of 100 mg of bupropion hydrochloride, are shown in Figure 3. The curves shown are representative of those obtained for each of the five subjects studied. In each case, the AUC values and the shapes of the curves calculated from data generated by both radioimmunoassay methods were similar.

Discussion

Previous studies of bupropion disposition in animals and humans have relied either on liquid chromatography (3) or the [3H]bupropion dextran-coated charcoal radioimmunoassay (4, 5). The immunoassay method represents roughly 100-fold improved sensitivity over the chromatographic method and is less cumbersome in its application to large numbers of samples. Nonetheless, we found the 125I-labeled p-hydroxybupropion radioimmunoassay method more convenient than the [3H]bupropion method. The polyethylene glycol separation method was much simpler than the dextran-coated charcoal method, requiring the use of a single disposable glass tube in the generation of each radioimmunoassay datum. The use of the high specific-activity radiiodinated ligand greatly reduced counting time, and assay cost was reduced considerably by eliminating the inherent expense of liquid scintillation counting. Moreover, the shorter half-life of 125I and the smaller volumes of liquid radioactive waste generated by the 125I

Table 2. Between-Assay Precision for [3H]Bupropion and 125I-Labeled pHydroxybupropion Radioimmunoassays

<table>
<thead>
<tr>
<th>Bupropion concn, μg/L</th>
<th>Added</th>
<th>Measured</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>[3H]Bupropion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>0.92</td>
<td>11.5% (n = 7)</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>10.3</td>
<td>5.1% (n = 7)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>93.9</td>
<td>5.6% (n = 7)</td>
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</tr>
<tr>
<td>1000</td>
<td>1113</td>
<td>7.8% (n = 3)</td>
<td></td>
</tr>
<tr>
<td>125I-Labeled p-hydroxybupropion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.00</td>
<td>1.18</td>
<td>12.2% (n = 4)</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>10.5</td>
<td>3.8% (n = 4)</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>98.8</td>
<td>6.7% (n = 4)</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>1185</td>
<td>8.8% (n = 4)</td>
<td></td>
</tr>
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</table>

Fig. 1. Bupropion radioimmunoassay standard curves generated with the [3H]bupropion dextran-coated charcoal method (A) or with the 125I-labeled p-hydroxybupropion polyethylene glycol method (B). Values are mean and SE of data from four separate curves with each method. B/B0, as discussed in text.

Fig. 2. Correlation of bupropion concentrations in human plasma samples as determined by the [3H]bupropion or 125I-labeled p-hydroxybupropion radioimmunoassay methods.
method should diminish radioactive waste-disposal problems, thereby making the analysis of bupropion plasma concentrations more widely available.

The two radioimmunoassay methods were comparable in most analytical variables, except that the use of the iodinated radioligand afforded somewhat greater sensitivity than that with \textsuperscript{3}H)bupropion. Moreover, the excellent agreement between bupropion concentrations in the five subjects, as determined by the two methods, demonstrated the suitability of the \textsuperscript{125}I-labeled \(\rho\)-hydroxybupropion method for direct plasma analyses.

References
8. Riggs DS, Guarnieri JA, Addelman S. Fitting straight lines when both variables are subject to error. Life Sci 22, 1305-1360 (1978).