Radioimmunoassay for Phencyclidine (PCP) in Serum

S. Michael Owens, James Woodward, and Michael Mayersohn

This accurate, sensitive radioimmunoassay for determining phencyclidine concentrations in serum specimens involves the use of anti-phencyclidine sera, 0.1 mL of serum specimen, an iodinated tracer, and a solid-phase separation. Phencyclidine metabolites do not show significant cross reactivity, but several phencyclidine analogs do cross react. Within-run coefficients of variation for human and dog serum ranged from 2.5 to 13% for concentrations from 2.0 to 500 μg/L. Day-to-day coefficients of variation for human and dog serum ranged from 4.3 to 16.7% for concentrations ranging from 2.0 to 90.0 μg/L. The sensitivity of the radioimmunoassay is <0.5 μg/L. Thirty serum specimens from two dogs given 1 mg of phencyclidine per kilogram body weight were analyzed by radioimmunoassay and a gas-chromatographic method. Nonparametric statistical comparison and linear regression showed that results from the two procedures correlate well ($r^2 = 0.952$). Concentration–time data from the two dogs are presented to illustrate the utility of the radioimmunoassay for examining phencyclidine disposition.

Additional Keyphrases: abused drug • drug assay • dogs • pharmacokinetics • cross reactivities

Phencyclidine (PCP) is known to be a dangerous drug of abuse. Yago et al. report that, of 145 consecutive patients seen at a psychiatric hospital emergency room, 43.4% of those admitted had PCP present in their serum (1). Other studies also suggest a high incidence of regional PCP use among certain groups (2, 3). Clinical studies indicate that PCP is responsible for psychotic, violent, and self-destructive behavior (4). Bailey and Guba found plasma PCP concentrations of 10 to 812 μg/L in 15 nonfatal intoxications (5). The few nonfatal PCP intoxications reported by Lin et al. are also within the concentration range found by Bailey and Guba; however, they report a blood PCP concentration of 2.7 mg/L in a person after a fatal overdose (6). A rapid, sensitive, and specific analytical method for PCP in blood specimens is desirable, to use in confirming the diagnosis of PCP-induced toxicity. In addition, such an analytical method is essential for the study of PCP disposition in animals and man, in order to develop rational approaches for the treatment of overdose.

Numerous analytical methods for PCP have been reported. PCP concentrations have been determined in blood, serum, or plasma by gas chromatography–mass spectrometry with chemical ionization or electron-impact selected-ion monitoring (6, 7), gas chromatography with a nitrogen sensitive detector (5, 8–10) or by radioimmunoassay (RIA) with tritium or iodine-125 labeled tracers (11–14). Each method of analysis has its advantages and disadvantages. However, the sensitivity, speed of analysis, and small sample volume required for RIA make it a desirable method of analysis.

The purpose of this study was to design and validate an RIA for PCP in serum specimens. The use of an $^{125}$I tracer, low cross-reactivity antibody, and a solid-phase second-antibody separation method proved both sensitive and specific. We also report PCP disposition studies in two dogs, to illustrate the utility of the assay.

Department of Pharmaceutical Sciences, College of Pharmacy, the University of Arizona, Tucson, AZ 85721.

1 Address correspondence to this author.

Received Jan. 21, 1982; accepted April 13, 1982.
Materials and Methods

Reagents and Standards

Standards. Phencyclidine hydrochloride, 1-(1-phenylcyclohexyl)-4-hydroxy piperidine, 4-phenyl-4-piperidinocyclohexanol, and 1,1-phenylethylcyclohexylmorpholine were obtained through the National Institute on Drug Abuse, Rockville, MD, and were provided by the Research Triangle Institute, Research Triangle Park, NC.

Serum standards and controls. PCP-free blood was obtained from mongrel dogs housed at the Division of Animal Resources, University of Arizona, and from human donors. Phencyclidine HCl in distilled water was added to pooled human or dog serum to yield concentrations equivalent to 1, 3, 10, 30, 60, and 100 μg of the free base per liter. Serum controls were prepared at concentrations of 2, 4, 5, 20, 25, 80, 90, 100, and 500 μg/L.

Radioligand. 125I-labeled PCP, synthesized from N-(4-hydroxy-2-phenethyl)-4-[(1-phenylidonil) cyclohexyl]-benzamidine, was provided by Roche Diagnostics, Nutley, NJ. The specific activity of the radioligand was approximately 50 kCi/mmol (15). The stock solution, stored in phosphate-buffered isonic saline at 4–8 °C, was protected from light.

Anti-PCP sera. Rabbit anti-PCP sera was provided by Roche Diagnostics.

RIA buffer. The pH 7.6 buffer contained, per liter, 50 mmol of tris(hydroxymethyl)aminomethane, 0.15 mol of NaCl, 1 g of bovine serum albumin (Pentex, Miles Laboratories, Elkhart, IN), and 2 g of sodium azide. It was stored at 4–8 °C.

Solid-phase second-antibody. A 20 g/L suspension of polymer-bound goat anti-rabbit immunoglobulins in phosphate-buffered saline was obtained from Roche Diagnostics. The suspension was diluted fourfold with RIA buffer and a 100-μL aliquot was used to precipitate antibody-bound radioactivity. The second-antibody preparation was added in excess of the anti-PCP sera concentration to assure complete precipitation of the rabbit anti-phencyclidine immunoglobulins.

Procedures

RIA. The radioligand was added to the RIA buffer to give a concentration of 235 ng/L, and 0.2 mL was pipetted into 10 × 75 mm glass test tubes. Then 0.1 mL of standard, control, or unknown serum was added to appropriately labeled tubes, in duplicate. Serum specimens with phencyclidine concentration >100 μg/L were diluted with blank serum so that concentrations would be within the range of the standard curve — i.e., 1–100 μg/L. The anti-PCP sera was diluted 15 000-fold (25–35% antibody binding in the absence of PCP) with RIA buffer, and 0.1 mL was added to all tubes except the tubes for nonspecific binding and total radioactivity. To the latter tubes, 0.1 mL of buffer was added. After vortex-mixing, the tubes were incubated at 30–35 °C for 1–2 h, then 0.1 mL of the solid-phase second antibody was added to all tubes except the tubes used to determine total counts. The tubes were further incubated at room temperature for 15 min with the second-antibody preparation and then centrifuged at 1000 × g for 5 min to precipitate antibody-bound radioactivity. The supernatant fluid was aspirated and the pellet washed with 0.8 mL of buffer. After centrifugation, the supernate was aspirated. The radioactivity in the pellet was counted in a gamma counter with 74.5% efficiency. The results were usually computer-calculated by the four-parameter logistic model (16, 17). However, we summarized the results for the average standard curve by using the logit-log transformation of the average counts from duplicate tubes (18).

Gas chromatography. We analyzed for PCP in serum specimens by a modification of the gas-chromatographic method of Bailey and Guba (5). Briefly, 1.0 mL of a 250 g/L solution of Na2CO3 was added to 1 mL of serum in a 10-mL siliconized (Aquasil; Pierce Chemical Co., Rockford, IL) glass culture tube. Two milliliters of hexane containing 1,1-phenylethylcyclohexylmorpholine as an internal standard was added to each tube, and the samples were shaken vigorously for 30 min, centrifuged, and the organic layer was removed and transferred to a second tube containing 2 mL of 0.1 mol/L H2SO4. The samples were shaken for 30 min and centrifuged, the organic layer was aspirated, and the remaining aqueous layer was alkalinized with 1 mL of the Na2CO3 solution. Three milliliters of fresh extraction solvent was added, and the tubes were shaken vigorously for 30 min and centrifuged. Two milliliters of the organic layer was transferred to a siliconized 10 × 75 mm culture tube and evaporated under a gentle steam of nitrogen at 40 °C. The residue was dissolved in 15 μL of methanol, and 1 μL was injected into the gas chromatograph with use of a splitless mode.

A Hewlett-Packard gas chromatograph (Model 5840A) equipped with a nitrogen-phosphorus detector and a Hewlett-Packard 25M methyl silicone capillary column were used for the analysis. Analysis for PCP was optimal under the following conditions: injection port temperature, 200 °C; detector temperature, 300 °C; oven temperature, programmed at 130 °C for 0.5 min, increased at a rate of 30 °C/min, then held at 210 °C for 6 min; carrier gas (helium) flow rate, 2.0 mL/min; air flow rate, 100 mL/min; hydrogen flow rate, 3.5 mL/min; make-up gas (helium) flow rate, 40 mL/min. Detector bead current was adjusted to give a 10-cm pen deflection at a 28 attenuation. Retention times for PCP and 1,1-phenylethylcyclohexylmorpholine were 6.25 and 6.95 min, respectively.

Serum unknowns. Phencyclidine hydrochloride (1 mg/kg as the free base) in physiological saline was administered to two normal mongrel dogs (18.8 and 22.3 kg) by intravenous bolus injection. Blood specimens were collected at specified times for 36 h thereafter. Serum specimens were refrigerated at 4–8 °C until analyzed. All analyses were completed within five days after specimens were collected.

Comparison of the RIA with the gas-chromatographic method. Thirty PCP-positive specimens from the PCP dosing study were analyzed by the RIA and the gas-chromatographic method in a blind fashion. We plotted and compared the results, using a linear regression model and nonparametric statistical tests.

Results and Discussion

Many of the reported analytical procedures for PCP in blood specimens have not been carefully validated. Accurate and reproducible analysis down to low (μg/L) PCP concentrations is essential for an accurate description of PCP disposition kinetics and for the diagnosis of PCP abuse.

We used RIA reagents originally developed for the analysis of PCP in urine and serum specimens (11, 12), but we completely redesigned the analytical procedure to provide a more sensitive system.

Sensitivity and reproducibility were adequate when serum specimens were incubated for 30 min with the PCP antisera at 30–35 °C; however, we routinely incubated the specimens with the PCP antisera for 1–2 h, because of the large number of specimens assayed at one time. Cold incubation, i.e., 4–8 °C, prolonged the incubation interval required and did not improve sensitivity.

We used 0.1 mL of serum in the assay because it gave the desired sensitivity and working range. However, use of 0.2 mL of serum approximately doubled the sensitivity without loss.

2 We could not obtain authentic PCP-containing human sera for examination.
Table 1. Reproducibility and Analytical Recovery for the RIA and Gas-Chromatographic Methods

<table>
<thead>
<tr>
<th>No. samples</th>
<th>PCP in serum, μg/L</th>
<th>Found (SD)</th>
<th>CV, %</th>
<th>Recovery, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>2.0 (0.25)</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>20.0 (1.02)</td>
<td>4.9</td>
<td>105</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>90.0 (4.08)</td>
<td>4.5</td>
<td>102</td>
</tr>
<tr>
<td>Dog serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>5.0 (0.23)</td>
<td>4.6</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>25.0 (1.47)</td>
<td>5.5</td>
<td>107</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>100.0 (2.36)</td>
<td>2.5</td>
<td>92.7</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>500.0 (21.6)</td>
<td>4.0</td>
<td>109</td>
</tr>
<tr>
<td>Human serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>2.0 (0.30)</td>
<td>16.7</td>
<td>90</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>20.0 (1.98)</td>
<td>10.7</td>
<td>93.0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>90.0 (3.91)</td>
<td>4.3</td>
<td>100</td>
</tr>
<tr>
<td>Dog serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>5.0 (0.65)</td>
<td>12</td>
<td>106</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>20.0 (1.10)</td>
<td>5.1</td>
<td>109</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>80.0 (5.33)</td>
<td>6.7</td>
<td>99.0</td>
</tr>
<tr>
<td>Gas-chromatographic method</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within-day variation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>4.0 (0.44)</td>
<td>12</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>20.0 (0.903)</td>
<td>4.3</td>
<td>105</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>80.0 (3.81)</td>
<td>5.0</td>
<td>94.9</td>
</tr>
<tr>
<td>Day-to-day variation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>4.0 (0.66)</td>
<td>15.7</td>
<td>105</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>20.0 (2.22)</td>
<td>11.4</td>
<td>97.0</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>80.0 (5.08)</td>
<td>6.6</td>
<td>96.0</td>
</tr>
</tbody>
</table>

Fig. 1. Comparison of results from analysis of PCP in dog serum by RIA and a gas-chromatographic method (GC) 

The solid line is a least squares fit of the data, $y = 1.10x - 2.7$ ($r^2 = 0.952$), and the stippled line is the line of identity, for comparison.
obtain the recently identified urinary PCP metabolite, 5-
[N-(1-phenylcyclohexy]amino]pentanoic acid, to test its
cross reactivity, but we would expect it to show little or none
because it lacks the piperidine portion of the molecule (19).
Heveran and Ward (12) found that the only significant
cross-reacting compounds were PCP analogs. This RIA for
PCP in serum will give accurate results only if these structural
analogs are not present. If this RIA is used for clinical or fo-
rensic purposes, exact identification must be performed by
a different method of analysis, such as gas chromatography-
mass spectrometry. However, only PCP or some of its analogs
should cross react with the antibody.

The within-day and day-to-day reproducibility and ana-
lytical recovery for the RIA in human serum and dog serum
were good (Table 1). The reproducibility of the gas chroma-
tographic method was also good (Table 1).
The solid-phase goat anti-rabbit antibody we used in this
RIA produced <0.5% non-specific binding; therefore, assay
noise and misclassification errors were kept to a minimum (21,
22).

The smallest measurable amount of PCP, determined at
2 SD below the mean of 15 determinations in the absence of
non-radioactive standard, was 28 pg (CV = 93%) per tube, an
amount equivalent to a concentration in serum of 0.28 μg/L.
However, values determined for sample concentrations were
accepted only if they were within a concentration range giving
a maximum standard error of <5% (23). The percent error of
a given concentration on the standard curve is affected by the
uncertainty in the response variable and the slope of the RIA
curve at that concentration. In 15 determinations the con-
centration at the low end of the standard curve at which the
percent error was equal to 5% ranged from 1.4 to 3.9 μg/L (x
= 2.3 μg/L, SD = 0.79). This strict criterion for the repro-
ducibility of each standard curve allowed a high degree of
confidence in the reported value for each unknown.

Although <1 μg of PCP per liter could be detected by the
gas-chromatographic procedure, a serum “background” peak
at the same retention time as PCP limited its sensitivity to 2–3
μg/L. Bailey and Guba (5) also noted such “background” in-
terference.

Results obtained on analysis of 30 PCP-positive serum
samples by RIA and the gas-chromatographic method were
compared by nonparametric statistical methods. Both the sign
test and Wilcoxon’s test accepted the hypothesis that there
is no difference between the two methods at a level of signif-
ance of α = 0.05. Least-squares regression analysis of RIA
vs gas chromatography values produced a slope of 1.10, y-
intercept of −2.7, and r² of 0.952 (Figure 1).

Because PCP can produce deleterious effects in humans,
we used the dog as an animal model to study the disposition
kinetics of PCP. We determined the serum concentrations of
PCP over time in two mongrel dogs that had received an
intravenous bolus dose of 1 mg of PCP per kilogram body weight
(Figure 2). The concentration–time data were best described
by a bi-exponential function with terminal elimination half-
lives of 2.3 and 4.1 h.

In conclusion, we found the sensitivity and reproducibility
of our RIA to be slightly better than that of our gas-chroma-
tographic method, while requiring only one-fifth the sample
volume and much less analysis time.

This study was supported by grant DA02680 from the National
Institute on Drug Abuse. We are particularly grateful to Mrs. Cynthia
Ward and Dr. Hans Hager of Hoffman-La Roche, Inc. for their gen-
erous cooperation and suggestions. We also thank Hoffman-La Roche,
Inc. for supplying the anti-PCP sera, 125I-labeled PCP, and solid-
phase second-antibody.

References
1. Yago, K. B., Pitta, F. N., Burgoyne, R. W., et al., The urban epi-