Ouabain Pharmacokinetics in Dog and Man

Determination by Radioimmunoassay

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With the technical assistance of William Findley, B.A.

SUMMARY
The pharmacokinetics of the relatively polar and rapidly acting cardiac glycoside ouabain (G-strophanthin) were studied in dogs and human subjects by the use of a newly devised radioimmunoassay technic. This method had high specificity and a sensitivity of less than 0.1 ng of ouabain per ml of plasma or urine. After administration of a single intravenous dose, the plasma ouabain concentration fell rapidly in both dogs and humans. After 7 hours, a phase of exponential decline was reached which had a half-life of 18 hours in dogs and 21 hours in normal human subjects. Repeated intravenous administration of ouabain to human subjects for 9 consecutive days resulted in the establishment of a plateau of plasma concentration and urinary excretion after 4–5 days, confirming plasma and urinary half-lives in the 19–24-hour range. The mean ratio of renal clearance of ouabain to that of creatinine was 0.81 in the human subjects. The plasma half-life of ouabain determined in these studies is in good agreement with previous observations of the half-life of dissipation of positive inotropy and of slowing of ventricular response to atrial fibrillation after ouabain administration.

Additional Indexing Words:
Cardiac glycoside
Ouabain-specific antibody

G-Strophanthin

Tritiated ouabain

A NUMBER of useful studies reported in recent years have defined the half-life and metabolic fate for the clinically important cardiac glycosides digoxin and digitoxin.1 Less complete data have been reported for the more polar and rapidly acting glycoside ouabain (G-strophanthin). A controversial aspect of previously reported investigations has been the half-life of the late phase of exponential decline of serum ouabain concentration. Estimates of the half-life of serum radioactivity after intravenous administration of tritiated ouabain (3H-ouabain) have ranged from as little as 5.5 hours in one study of normal human volunteers2 to 50 hours in another series of subjects with normal cardiac, renal, and liver function.3 The half-life of dissipation of the positive inotropic effect, on the other hand, has been estimated at 22 hours.4 Slowing of the ventricular response to atrial fibrillation after a single intravenous dose of ouabain was found to be about 50% dissipated after 1 day.5

The purpose of the present study was to delineate the plasma kinetics of ouabain by means of a radioimmunoassay with sufficient sensitivity to measure picomolar concentrations of unlabeled ouabain in biologic fluids.6 The plasma disappearance curve after single intravenous doses of ouabain was determined in dogs and normal human subjects. Plasma concentrations and urinary excretion were also
studied in normal human subjects under steady-state conditions produced by daily intravenous injections of ouabain over a 9-day period.

Methods

Assay Method

Details of the assay method are presented elsewhere. Ouabain-specific antiserum with an average intrinsic association constant (Ko) of $1.3 \times 10^9$ M$^{-1}$ and restricted heterogeneity of binding site affinities was obtained by serial injections of rabbits with ouabain covalently coupled to human serum albumin through an extended side arm of poly-DL-alanine. Suitable amounts of $^3$H-ouabain (specific activity 11.7 Ci/mmole, New England Nuclear Corporation, Boston, Massachusetts) and ouabain-specific antibody were added directly to plasma samples. For urine assays, small aliquots of urine (10–50 µl) were added. Following a brief period of equilibration of labeled and unlabeled ouabain with antibody-binding sites, activated charcoal coated with molecular-weight 80,000 dextran was added. Free ouabain was selectively bound to the charcoal and centrifuged, allowing antibody-bound $^3$H-ouabain to be decanted into a toluene-detergent base scintillation fluid and counted in a liquid scintillation spectrometer. Quenching variation was corrected by the use of a $^{226}$radium external standard. Unknown samples were estimated by comparison with a standard curve constructed with known amounts of the same ouabain preparation (Eli Lilly) used in the experimental animals and human volunteers. These standards were prepared using plasma obtained prior to ouabain infusion.

The sensitivity of the method allowed quantitation of concentrations as low as 0.05 ng/ml (fig. 1). Replicate measurement of 10 identical samples yielded a standard deviation of ±5% or less over the range from 0.05 to 10 ng/ml. Control plasma and urine samples to which known amounts of ouabain had been added were stored in the dark at 4° for 30 days to test stability and recovery under these conditions. These samples yielded ouabain concentrations by radioimmunoassay which were within 5% of values derived from a freshly prepared standard curve over the range 0.1 to 10 ng/ml. Specificity studies showed no interference from endogenous steroid concentrations well above those occurring physiologically. The rhamnose moiety of ouabain did not produce measurable displacement of $^3$H-ouabain from the antibody-binding site when present in 35,000-fold molar excess. False-positive values were not encountered in subjects not receiving radioactive drugs.

![Figure 1](https://circ.ahajournals.org/content/full/41/4/1177/F1.large.jpg)

**Figure 1**

Semilogarithmic plot of standard curve. Range of duplicate determinations shown. The arrow on the vertical axis indicates percent antibody-bound $^3$H-ouabain in the absence of unlabeled ouabain. 1 ng of $^3$H-ouabain tracer was used.

Canine Studies

Under intravenous methohexitol (Brevital) anesthesia (1 mg/kg), a polyethylene catheter was inserted into the external jugular vein of seven 16–21-kg mongrel dogs. A control sample of blood was obtained from each dog. Ouabain, 0.025 mg/kg in 10 ml of normal saline, was then infused into a foreleg vein over a period of 30 sec. The jugular venous catheter, kept patent with heparinized saline, was used to obtain blood samples at 1/30, 1/12, 1/6, 1/4, 1/2, 1, 2, 3, 5, 7, 12, 24, 30, 36, and 48 hours after the injection. The dogs were awake and ambulatory after placement of the jugular venous catheter.

Human Studies

Three healthy male subjects, ages 26 to 35 years, without renal, hepatic, cardiac, or other disease, volunteered for this study. Table 1 lists anthropometric and creatinine clearance data for these subjects. Fully informed consent was obtained from each subject. Each was given 0.5

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Creatinine clearance (liters/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>190</td>
<td>82</td>
<td>196</td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>180</td>
<td>73</td>
<td>193</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>168</td>
<td>73</td>
<td>146</td>
</tr>
</tbody>
</table>
mg of ouabain intravenously via an antecubital vein; blood samples were subsequently obtained from the contralateral arm at 1/12, 1/4, 1/2, 1, 2, 4, 7, 9, 12, 24, 33, and 48 hours after the infusion. This experimental protocol was carried out on two occasions with an interval of 2 months between studies. Ouabain and creatinine concentrations in urine samples collected between 7 and 9 hours after ouabain administration and in blood samples drawn at 7 and 9 hours were used to calculate ouabain-to-creatinine renal clearance ratios. Creatinine concentrations were determined with an alkaline picrate assay.7

In addition to measuring plasma disappearance after a single dose of ouabain, experiments designed to achieve a steady state were carried out. Measurements were made during a period of daily intravenous administration of 0.25 mg of ouabain for 9 consecutive days. Blood samples were obtained just before each daily dose and at 24 and 48 hours after the last intravenous dose of ouabain. All urine was collected for a period of 12 days, including the 9 days of ouabain administration and the 3 days subsequent to the last dose.

Samples

All blood samples were drawn into heparinized tubes, centrifuged, and the plasma immediately separated and stored at 4°C. Urine volumes were measured and aliquots stored at 4°C. Ouabain concentration determinations were carried out within 10 days in all cases. As noted above, control experiments showed no significant change in the concentrations measured under the storage conditions used for periods of up to 1 month. Heparinized plasma samples gave results identical to those obtained with serum samples simultaneously collected.

Statistics

Statistical evaluation, including least-squares linear regression analyses, were performed by conventional methods8 with the aid of an IBM 360-65 time-sharing computer.

Results

Canine Studies

Plasma ouabain concentration after intravenous injection fell rapidly at first, then reached a stable exponential decay phase after 7 hours. Figure 2 is a semilogarithmic plot of mean plasma ouabain concentration against time following the intravenous administration of a single dose of 0.025 mg/kg of ouabain to each of seven dogs. The initial very rapid decline in plasma ouabain concentration had a half-life of about 3 min and probably represents mixing in the intravascular compartment as well as the initial phase of tissue uptake. This was followed by a period of about 6 hours characterized by a gradually decreasing rate of ouabain disappearance. This phase was thought to represent primarily tissue uptake and distribution, with an additional contribution from excretion. About 7 hours after ouabain administration, a phase of exponential decline of plasma concentration with a half-life of 18 hours was established which remained constant up to 48 hours. During this late phase between 7 and 48 hours after ouabain administration, plasma half-lives in the individual dogs ranged from 17 to 24 hours.

Human Studies

Plasma ouabain kinetics following a single intravenous injection in normal human subjects were very similar to the canine data. Figure 3 is a semilogarithmic plot of mean plasma ouabain concentration following the intravenous administration of a single 0.5-mg dose to each of three subjects on two
OUABAIN PHARMACOKINETICS

Semilogarithmic plot of mean human plasma ouabain concentration vs time. Mean values for six experiments are plotted. The half-life of plasma ouabain concentration between 7 and 48 hours is 21 hours (r = 0.99 for least-squares plot).

occasions separated by 2 months. The plasma ouabain concentrations at each interval after ouabain administration were less in the humans than in the dogs, as would be anticipated from the relatively smaller doses given on an mg/kg basis. Nevertheless, the shapes of the curves in the two species were very similar. The half-life of mean plasma ouabain concentration for the late phase of exponential decline as determined by least-squares linear regression analysis was 21 hours. Half-lives in the individual subjects ranged from 18.4 to 25.0 hours, with a mean value of 21.8 ± 2.0 hours (table 2).

Determination of simultaneous ouabain and creatinine clearances during the late phase of exponential decline yielded a mean ouabain-to-creatinine renal clearance ratio of 0.81, with a range from 0.73 to 0.90 in individual subjects, as shown in table 2.

In addition to these studies following a single dose, plasma levels and urinary excretion of ouabain were measured in further experiments designed to produce a steady state. The same three subjects were given 0.25 mg of ouabain intravenously at 24-hour intervals for 9 consecutive days. Figure 4, top, shows the mean plasma ouabain concentration immediately prior to each dose. A plateau was established at 4–5 days. Mean values for 24-hour urinary excretion of ouabain during the same period are also shown in figure 4, bottom. A rise in ouabain recovered in the urine was observed until the fourth or fifth day, when a plateau was established concomitant with the plasma level plateau. Mean

Table 2

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma Half-life (hr)</th>
<th>Corr coeff*</th>
<th>Ouabain: creatinine (renal clearance ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25.0</td>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td>1†</td>
<td>18.4</td>
<td>0.97</td>
<td>0.90</td>
</tr>
<tr>
<td>2</td>
<td>21.2</td>
<td>0.97</td>
<td>0.80</td>
</tr>
<tr>
<td>2†</td>
<td>23.2</td>
<td>0.98</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>22.0</td>
<td>0.99</td>
<td>0.73</td>
</tr>
<tr>
<td>3†</td>
<td>20.8</td>
<td>0.96</td>
<td>0.73</td>
</tr>
<tr>
<td>Mean</td>
<td>21.8 (± 2.0 SD)</td>
<td></td>
<td>0.81</td>
</tr>
</tbody>
</table>

*For best linear fit by least-squares linear regression analysis of plot of log ouabain concentration vs time between 12 and 48 hr.

†The second of the two studies in each subject was carried out 2 months after the initial experiment.

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plasma concentration after establishment of a steady state (days 5-9) varied from 0.41 to 0.64 ng/ml among the individual subjects (table 3). Individual mean 24-hour urinary recoveries of ouabain during the steady state varied from 86 to 94 μg with a mean urinary recovery for the group of 91 μg (37%) of the 250 μg administered each day. Establishment of a plateau of plasma concentration and of urinary excretion occurred between the fourth and fifth day in each individual. Following the last dose of ouabain, plasma concentration and urinary excretion declined with half-lives of about 20 hours.

Discussion

Previous studies have employed tracer doses of $^3$H-ouabain given to experimental animals and human subjects.2, 3, 9-11 Renal clearance has been found to be the predominant route of excretion, with 45-63% of administered radioactivity recovered in urine over the initial 24 hours.2 Biliary excretion has been estimated at only 2-8% for the initial 24 hours.2 Compared with normal controls, patients with renal insufficiency have been reported to maintain higher serum levels of the drug with prolongation of the half-life of the late phase of exponential decline.3, 10 Impaired hepatic function, on the other hand, did not alter serum half-life or urinary excretion of $^3$H-ouabain.3 Hyperthyroidism shortened the serum half-life and increased the rate of urinary excretion of ouabain, while hypothyroidism had opposite effects.11

Prior estimates of plasma ouabain half-life in man, limited by the time interval during which plasma radioactivity could be accurately measured after administration of $^3$H-ouabain, have varied widely. Marks et al.2 estimated the half-life at 5.5 hours in human subjects, but cautioned that their data extended for a relatively short period (about 2 hours) after ouabain administration. Estimates of 50 hours based on reliable counts up to 6 hours3 and 13.9 hours based on measurements for 24 hours10 after $^3$H-ouabain infusion have also been reported for normal control patients and human volunteers, respectively. The radioimmunoassay of plasma ouabain used in our studies, with a sensitivity of less than 0.1 ng/ml, allowed accurate determination of plasma ouabain concentration for more than 48 hours after a single subdigitalizing dose of 0.5 mg. This method yielded a mean plasma ouabain disappearance curve with a late exponential half-life of 21 hours in normal human subjects, as shown in figure 3. This value was similar to the mean plasma half-life of 18 hours observed in our canine experiments (fig. 2). It is of interest that a similarity between canine12-14 and human15 plasma digoxin half-lives has also been observed.

It should be noted that plasma and urinary concentrations of ouabain determined by our method represent maximum possible concentrations of native ouabain present in a sample. Biotransformation, if it occurs, might yield products capable of displacing $^3$H-ouabain from the antibody-binding site, thereby increasing the apparent ouabain concentration. A similar problem exists, of course, when ouabain concentrations are determined by measurement of tritium counts after administration of $^3$H-ouabain.

Another approach to estimating the plasma half-life of a cardiac glycoside (or other drug), the concentration of which declines in an exponential fashion, is to determine the length of time necessary to establish a plasma concentration plateau with administration of the drug at regular intervals. The shape of such a curve is described by the general equation:

$$%P = (1 - e^{-kt}) \times 100$$

*Table 3*

**Plateau Values during Daily Intravenous Administration of Ouabain**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma conc (ng/ml)*</th>
<th>Urinary excretion (μg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean†</td>
<td>Range†</td>
</tr>
<tr>
<td>1</td>
<td>0.64</td>
<td>0.62-0.65</td>
</tr>
<tr>
<td>2</td>
<td>0.47</td>
<td>0.44-0.48</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
<td>0.40-0.43</td>
</tr>
<tr>
<td>Mean</td>
<td>0.51</td>
<td></td>
</tr>
</tbody>
</table>

*24 hours after last dose.
†Days 5 to 9.
where \( %P \) = percent attainment of the steady-state plateau; \( k \) = the rate constant of elimination; \( e \) = the natural logarithm base; and \( t \) = time.\(^{16} \) After one half-life, \( %P = 50\% \). Similarly, after two, three, four, or five half-lives, \( %P \) is 75, 87.5, 94, and 97\%, respectively. This has been experimentally documented for \(^3\)H-digoxin in humans\(^{17}\) and dogs.\(^{18}\) In our steady-state experiments, establishment of a plasma level and urinary excretion plateau after 4–5 days, as shown in figure 4, indicates a half-life of elimination in the 19–24-hour range, in good agreement with the mean plasma concentration half-life of 21 hours observed after single doses of ouabain.

Recovery of radioimmunoassayable ouabain from the urine of human subjects during the steady-state plateau averaged only 37\% of the intravenously administered daily dose (fig. 4). The remaining 63\% presumably underwent biotransformation to a form which interacted less strongly with the antibody-binding sites and/or elimination from the body by a nonrenal route. Further studies to clarify this issue are underway.

The plateau principle is applicable not only to the time course of increase in plasma concentration or excretion of a cardiac glycoside, but also to pharmacologic effects. Gold and DeGraff\(^{19}\) measured the ventricular response to atrial fibrillation during the daily administration of digitalis leaf and observed a plateau after about 4–5 weeks, compatible with an excretory half-life of about 6 days. Similar ventricular response data for digitoxin showed a plateau at 3 weeks\(^{20}\) compatible with a half-life of about 4.2 days, in good agreement with the 4.8-day half-life of plasma level decline observed by Lukas.\(^{21}\)

Serial quantitation of such effects as positive inotropy and slowing of the ventricular response to atrial fibrillation after single doses has been employed to determine the effective pharmacologic half-life of cardiac glycosides.\(^{4, 5}\) Weissler et al., using systolic time intervals from carotid pulse tracings as an indication of left ventricular contractility, determined the half-lives of dissipation of the positive inotropic effects of digoxin and digitoxin to be 33 and 102–112 hours, respectively.\(^{4}\) These values are in good agreement with direct estimates of the half-lives of these drugs in the body.\(^{15, 21}\) The half-life of ouabain's inotropic effect was determined to be 22 hours in normal human subjects by serial systolic time intervals.\(^{4}\) Similarly, Gold et al. observed the slowing of the ventricular response to atrial fibrillation after a single dose of ouabain to be about 50\% dissipated after 1 day.\(^{5}\) Replotting these data\(^{5}\) as change in heart rate (log scale) versus time, a straight-line function with a half-life of 23 hours is obtained (fig. 5). Thus the 21-hour half-life of plasma ouabain concentration in normal man, as determined by radioimmunoassay, is in good agreement with prior estimates based on measurement of pharmacologic effects.

From a clinical standpoint, it is important to note that the total amount of ouabain in the body, and hence the risk of toxicity, in a patient placed on any regular dosage schedule without a loading dose (irrespective of the interval between doses) will continue to rise for the initial 4–5 days if renal function is normal. Accumulation will proceed for an even longer time in the presence of a
prolonged excretory half-time due to renal functional impairment.

References


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