Plasma and Tissue Radioactivity of $^3$H-digitoxin in Dogs with Experimental Acute Renal Failure

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ABSTRACT. Twenty-two dogs were divided into 3 groups; a control (C) group of sham-operated dogs, a bilateral ureter ligation (L) group, and a bilateral nephrectomy (N) group. $^3$H-digitoxin was given to these dogs in a single intravenous administration, and the kidney involvement in the metabolism and excretion of digitoxin was investigated on the basis of radioactive indications in plasma and the tissue of the heart, liver and kidney. During the 24 hr observation, the radioactivity from the plasma dichloromethane (CH$_2$Cl$_2$)-soluble fraction was significantly higher in both the L and N groups than in the C group. The plasma elimination half-life of the CH$_2$Cl$_2$-soluble fraction in the C group was about 9.3 hr. The L and N groups had a significantly longer (about 2 times) plasma elimination half-life than the C group. The radioactivity in canine heart and liver were almost the same in each group 24 hr after dosing. They were lowest in the C group, intermediate in the L group and highest in the N group. In the C and L groups, the radioactivity was higher in the kidney than in the heart or liver. The radioactivity in the kidney was higher in the C group than in the L group. There was a significant positive correlation between the tissue radioactivity and the plasma CH$_2$Cl$_2$-soluble fraction radioactivity. This indicates that the kidney may affect digitoxin metabolism and excretion in the dog. Also, patients should be evaluated regarding not only liver function but also renal function.—KEY WORDS: cardiac glycoside, digitalis, digitoxin, renal failure.


In dogs, digitoxin, the more lipid-soluble glycoside, is extensively metabolized by the liver [10]. Since the liver is an important site in digitoxin disposition, caution is advised when administering the drug in dogs with hepatic diseases [16, 17]. Although digitoxin and its cardioactive and cardioinactive metabolites are excreted in the bile and urine after dosing, most of the excreted materials are water-soluble cardioinactive metabolites of digitoxin [7, 12, 16, 21]. As there is little urinary excretion of the unchanged substance and cardioactive metabolites, it seems that pharmacokinetics of digitoxin may be little affected in renal diseases. Also in patients with renal malfunctions, the use of digitoxin may not present clinical problems, if the liver function is normal.

In man, it has been reported that the pharmacokinetics of digitoxin is not altered in patients with impaired renal function [24]. Storstein [24] observed that serum digitoxin concentrations are decreased and the elimination half-life is shortened in uremic patients, although the renal excretion of the drug is reduced. Because the biotransformation of digitoxin in dogs is obviously different from that in man [8, 11, 16, 23, 24], confusion may result from the extrapolation of the data from man to the dog [11].

Studies of the effect of the kidney on digitoxin and its cardioactive metabolites after dosing have been scanty in dogs. Furthermore, it is not clear if renal malfuncion has a significant influence on pharmacokinetics of digitoxin. The present study was
undertaken to determine the role of the kidney in $^3$H-digitoxin metabolism and excretion in dogs with experimental acute renal failure.

**MATERIALS AND METHODS**

Experiments were performed in 22 apparently healthy adult mongrel dogs of either sex, weighing between 7.0 and 20.5 kg. Experimental acute renal failure was produced by bilateral ureter ligation or nephrectomy. After these dogs were anesthetized with pentobarbital-Na, a midline anterior abdominal incision was made. In 7 dogs, bilateral ureters were doubly ligated at the middle of the ureter (L group). The other 7 dogs underwent bilateral total nephrectomy (N group). The 8 control dogs were incised in the abdomen, then the incisions were closed. The kidney and ureter were left intact in these control dogs (C group). Pentobarbital-Na was used for supplemental anesthesia as required during the experimental period in all dogs.

Digitoxin was intravenously administered 5 to 6 hr after the operation. Each dog received 4 µCi/kg of tritium-labeled digitoxin (New England Nuclear Corporation) and 0.020 mg/kg of cold digitoxin as carrier. Venous blood samples from each animal were drawn before the operation, and at 0, 1, 3, 6, 12, 18 and 24 hr after intravenous injection of the drug.

In the C group, a catheter was placed in the urinary bladder via the urethra. Urine samples were collected at 12 hr intervals following administration of the drug, and then the total volume was measured.

The heart, liver and kidney were removed to determine the radioactivity in the tissues 24 hr after the intravenous injection of $^3$H-digitoxin.

**PLASMA AND URINE RADIOACTIVE MEASUREMENTS:** Each sample was extracted with dichloromethane (CH$_2$Cl$_2$) to separate water-soluble metabolites from CH$_2$Cl$_2$-soluble metabolites and the parent compound. One ml of plasma or urine was poured into a separatory funnel, then 10 ml of CH$_2$Cl$_2$ were added, and the mixture was shaken vigorously by hand for a few minutes. This extraction step was repeated once. After separation of the two phases, the CH$_2$Cl$_2$ fraction was pooled, evaporated to dryness, then redissolved with 1.0 ml methyl alcohol and transferred to a scintillation counting vial containing a scintillation cocktail consisting of 7.0 g of 2, 5-diphenylxazole (PPO), 0.3 g of 2, 2'-Phenylen-bis-[5-phenylxazole] (POPOP), and 100 g of naphthalene in dioxane to make one liter. The CH$_2$Cl$_2$-insoluble residue (1 ml) was added to 2.0 ml of distilled water and 0.5 ml of this water fraction was pipetted into a counting vial. The radioactivity of the CH$_2$Cl$_2$-soluble and insoluble fractions was counted in an Aloka LSC-653 liquid scintillation counter. Quenching was corrected using automatic external standardization.

**TISSUE RADIOACTIVE MEASUREMENTS:** Specimens of the left ventricle, right ventricle, liver and kidney were cut into small pieces. The blood was rinsed out with physiological NaCl solution, then the pieces were gently blotted. The individual specimens were weighed prior to drying (mean wet weight: heart, 86 mg; liver, 83 mg; kidney, 104 mg). The total radioactivity of these tissue specimens was determined according to the oxygen flask combustion method as described by Oliverio et al. [19].

**MEASUREMENTS OF RENAL FUNCTION:** The plasma creatinine concentration and blood urea nitrogen (BUN) were determined by standard clinical laboratory techniques. Samples were obtained during the preoperative period, just prior to the dosing, and 24 hr after the drug administration.
RESULTS

A semi-log plot of the disappearance of the mean radioactivity in the plasma in the 3 groups after a single intravenous \(^3\)H-digitoxin dose is shown in Fig. 1.

In the C group, the plasma CH\(_2\)Cl\(_2\)-soluble and insoluble radioactive concentrations declined rapidly during the first few hours after dosing. After 12 hr, the radioactivity declined in a slow and exponential fashion. The plasma elimination half-life was calculated using a least squares linear regression analysis of radioactivity at times 12–24 hr after injection of the drug. In the 8 control dogs, the plasma elimination half-life of the CH\(_2\)Cl\(_2\)-soluble fraction ranged from 6.0 to 11.0 hr, with a mean of 9.35±0.54 (±SE) hr. The radioactivity in the CH\(_2\)Cl\(_2\)-insoluble fraction was higher than the CH\(_2\)Cl\(_2\)-soluble fraction at each sampling time. There was a constant relationship between the radioactivities in the plasma CH\(_2\)Cl\(_2\)-soluble and insoluble fractions from 3 to 24 hr. The latter was about 2.5 times as great as the former, although the absolute radioactivity of both fractions decreased gradually.

The analysis of the 24 hr urine samples at 12 hr intervals showed that most of the radioactivity was eliminated as the CH\(_2\)Cl\(_2\)-insoluble fraction (Table 1), while less than 1% of the administered drug was excreted as CH\(_2\)Cl\(_2\)-soluble fraction. The urinary excretory rate for the radioactivity in the CH\(_2\)Cl\(_2\)-soluble and insoluble fractions was greater during the first 12 hr after the dosing. Only 6% of the radioactivity excreted in urine was from the CH\(_2\)Cl\(_2\)-soluble fraction.

In comparison with the C group, the L and N groups had significantly higher plasma radioactivity in the CH\(_2\)Cl\(_2\)-soluble fraction, although the high radioactivity in the L and N groups was gradually reduced. There

<table>
<thead>
<tr>
<th>Collection Period</th>
<th>CH(_2)Cl(_2)-Soluble Fraction(^a)</th>
<th>CH(_2)Cl(_2)-Insoluble Fraction(^a)</th>
<th>CH(_2)Cl(_2)-Soluble Fraction % in Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–12 hr.</td>
<td>0.59±0.04(^b)</td>
<td>10.04±1.18</td>
<td>5.9±0.7</td>
</tr>
<tr>
<td>12–24 hr.</td>
<td>0.25±0.03</td>
<td>5.20±1.40</td>
<td>6.3±1.4</td>
</tr>
<tr>
<td>Total</td>
<td>0.84±0.03</td>
<td>15.24±2.18</td>
<td>5.9±0.8</td>
</tr>
</tbody>
</table>

\(^a\) Expressed as % of dose.
\(^b\) Mean±S.E.
Table 2. Tissue radioactivity and correlation coefficients between tissue and plasma levels

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Ureter Ligation</th>
<th>Nephrectomy</th>
<th>Correlation with Plasma (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right Ventricle</td>
<td>66.2±13.9</td>
<td>104.3±29.0</td>
<td>178.0±21.0</td>
<td>0.7428</td>
</tr>
<tr>
<td>Left Ventricle</td>
<td>69.1±13.1</td>
<td>104.8±28.1</td>
<td>194.5±22.9</td>
<td>0.7404</td>
</tr>
<tr>
<td>Liver</td>
<td>63.9±7.5</td>
<td>123.9±33.7</td>
<td>170.0±24.0</td>
<td>0.8553</td>
</tr>
<tr>
<td>Kidney</td>
<td>596.3±107.9</td>
<td>300.5±88.3</td>
<td>—</td>
<td>0.7599 (C-group)</td>
</tr>
<tr>
<td>Plasma</td>
<td>2.5±0.2</td>
<td>6.5±1.1</td>
<td>6.9±1.0</td>
<td>0.7362 (L-group)</td>
</tr>
</tbody>
</table>

a) Radioactivity expressed as ×10² dpm/g wet weight.
b) CH₂Cl₂-soluble fraction dpm/ml, 24 hours after the dosing.
c) Mean±S.E.
d) Not done.
e) Significantly different from control.
f) Significantly different from nephrectomy.
†: 0.05<P<0.1, *: P<0.05, **: P<0.01, ***: P<0.001.

was a significant difference in the mean plasma elimination half-life between the C and the L and N groups (L group: 17.3±3.2, range 12.0–32.2; N group: 19.6±1.9, range 13.7–27.7 hr). In the L group, one dog that had an elimination half-life of 94.3 hr, was excluded from the mean half-life calculation. The radioactivity in the plasma CH₂Cl₂-insoluble fraction in the L and N groups did not decrease, but remained significantly higher than that of the C group (Fig. 1).

The distribution of radioactivity expressed as dpm/gram wet weight of tissue in 3 groups is shown in Table 2.

The radioactivity in the right and left ventricles were almost the same. The radioactivity in the heart and liver was similar.

The heart and liver of the N group had a significantly higher radioactivity compared to the C group. The mean radioactivities in the L group were also higher than those in the C group although the differences are not statistically significant. In the C and L groups the radioactivity was higher in the kidney than in the heart or liver, and the kidney radioactivity tended to be higher in the C group than in the L group.

Table 2 also shows the correlation between the tissue radioactivity and the radioactivity in the plasma CH₂Cl₂-soluble fraction. There was a significant linear correlation between the individual tissues and the plasma concentrations in all groups, when one clearly aberrant case in each of the three groups was excluded.

The mean ratio of heart or liver to plasma radioactivity for the C and L groups was about 20/1, and about 35/1 for the N group, and the differences are significant. The kidney to plasma ratio in the C and L groups was about 200/1 and 50/1, respectively.

Experimental acute renal impairment induced by the ureter ligation or nephrectomy was evidenced by a post-operative increase in plasma creatinine just before dosing to 138% (L group) and 154% (N group) of the preoperative level. The BUN also increased to 155 and 176%. At the end of the experiment, the mean plasma creatinine of the L and N groups increased further to 350 and 342% respectively, and the BUN to 349 and 398%. In the C group, the plasma creatinine and BUN stayed within a normal range during the experimental period. In these parameters, there were significant differences between the C and the L or N
Table 3. Plasma or serum elimination half-life of digitoxin in dogs

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of Animals</th>
<th>Dose (µg/kg)</th>
<th>Route of Administration</th>
<th>Duration of Observation (hr)</th>
<th>Half-Life T 1/2 (hr)</th>
<th>Analytical Method</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965 Katzung B.G. et al.</td>
<td>4</td>
<td>20–50</td>
<td>iv&lt;sup&gt;a)&lt;/sup&gt;</td>
<td>8</td>
<td>14</td>
<td>&lt;sup&gt;3&lt;/sup&gt;H&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Amount remaining in body</td>
</tr>
<tr>
<td>1973 Breznock E.M.</td>
<td>6</td>
<td>44</td>
<td>iv</td>
<td>120</td>
<td>48.67±7.24</td>
<td>&lt;sup&gt;3&lt;/sup&gt;H</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>50</td>
<td>po&lt;sup&gt;b)&lt;/sup&gt;</td>
<td>216</td>
<td>49.40±6.32</td>
<td>&lt;sup&gt;3&lt;/sup&gt;H</td>
<td>Cardiac disease</td>
</tr>
<tr>
<td>1976 Bluschke V. et al.</td>
<td>4</td>
<td>388±13</td>
<td>iv</td>
<td>8–24</td>
<td>9.3 ±0.7</td>
<td>RIA&lt;sup&gt;90&lt;/sup&gt;</td>
<td>4 µg/kg/min</td>
</tr>
<tr>
<td></td>
<td>24–96</td>
<td>49.6 ±6.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1978 De Rick A. et al.</td>
<td>4</td>
<td>po</td>
<td>30</td>
<td>13.7</td>
<td>9.5–19.0</td>
<td>RIA</td>
<td>100 µg/kg/12 hr ×2 days</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>po</td>
<td>30</td>
<td>12.6</td>
<td>9.0–15.5</td>
<td>RIA</td>
<td>30–60 µg/kg/12 hr ×17 days</td>
</tr>
<tr>
<td>1976 Hamline R.L.</td>
<td>8</td>
<td>iv</td>
<td>24</td>
<td>8</td>
<td>7–12</td>
<td>RIA</td>
<td></td>
</tr>
<tr>
<td>1979 Amlie J.P. et al.</td>
<td>8</td>
<td>iv</td>
<td>24</td>
<td>5.7</td>
<td>3.8–7.4</td>
<td>RIA</td>
<td>2000 µg/head</td>
</tr>
<tr>
<td>1979 Shah G. et al.</td>
<td>5</td>
<td>150</td>
<td>iv</td>
<td>34</td>
<td>6.69±1.97</td>
<td>4.67–9.33</td>
<td>RIA</td>
</tr>
<tr>
<td>1981 Peters D.N. et al.</td>
<td>5</td>
<td>iv</td>
<td>24</td>
<td>8.17±3.40</td>
<td>4.23–11.27</td>
<td>RIA</td>
<td>1000 µg/head</td>
</tr>
</tbody>
</table>

<sup>a)</sup> Intravenous.
<sup>b)</sup> Per os.
<sup>c)</sup> Tritium radioactivity.
<sup>d)</sup> Radioimmunoassay.

groups at 0 and 24 hr after the dosing.

**DISCUSSION**

Digoxin is excreted primarily by the kidney, mostly as an unchanged original glycoside [9, 13–15]. Consequently, it has been generally accepted that digoxin should be used with caution in dogs with renal failure [15] or nephritis [8]. However, the pharmacokinetics of digitoxin in dogs is not well known.

In the present study, the plasma elimination half-life of the radioactivity in the CH₂Cl₂-soluble fraction (digitoxin and its cardioactive metabolites) averaged about 9 hr in the C group after a single intravenous administration of <sup>3</sup>H-digitoxin. Similar values of plasma half-life were obtained in a previous study with the digitoxin radioimmunoassay method [16] and were reported elsewhere [1, 5, 8, 12, 20, 22] (Table 3). In studies by Breznock [4] and Bluschke et al. [3], higher values were found. In our study, about 16% of the dose was excreted in urine during the first 24 hr after the dosing. This excretion rate was smaller than the values reported by Geiling (41%/day) [7] and Katzung et al. (25.8%/8 hr) [12]. However, most (about 94%) of the excreted radioactivity was a CH₂Cl₂-insoluble fraction in the C group. This is in agreement with the results obtain by Geiling [7], Katzung et al. [12] and Russell et al. [21].

It is not clear whether the pharmacokinetics of digitoxin is altered in dogs with renal dysfunction. However, in the present experiment with bilateral ureter ligation and nephrectomy, the plasma CH₂Cl₂-soluble and insoluble radioactivity was significantly higher and its elimination half-life was significantly longer than those in the C group. The radioactivity in the heart and liver was also increased by ureter ligation or nephrectomy.

The high plasma concentration of the CH₂Cl₂-insoluble fraction itself seems to cause no significant problems in clinical
situations (digitoxin therapy), because this fraction is a water-soluble cardioactive metabolite of digitoxin. However, Katzung et al. [12] have indicated that these watersoluble metabolites excreted into bile are hydrolyzed to relatively nonpolar chloroform-soluble products (e.g. digitoxin and its derivatives) during their passage through the canine gut, and can be absorbed. On the other hand, there is no evidence for biotransformation of water-soluble digitoxin metabolites back into digitoxin or any other lipid-soluble cardioactive substances [18].

The radioactivity in the plasma CH₂Cl₂-soluble fraction increased in the dogs with bilateral ureter ligation or nephrectomy. However, in the C group, the urinary excretion of the labeled CH₂Cl₂-soluble fraction (not the CH₂Cl₂-insoluble fraction), was only 1% of the dose during the first 24 hr. This is in agreement with the results obtained by St. George et al. [23]. This minute excretion is too small to account for the higher plasma CH₂Cl₂-soluble radioactive concentration or its longer half-life in the L and N groups.

The alteration of these parameters in the L and N groups may be partly explained by the higher radioactivity in the kidney, the decrease of plasma protein-drug binding, the alteration of tissue distribution, and so forth. St. George et al. [23] and Friedman et al. [6] found that radioactivity in the kidney was higher and its disappearance was slower than it was in the heart or liver in the dog. So they suggested that the kidney may destroy more digitoxin than it excretes.

About 90% of the digitoxin in the blood is bound to plasma albumin [2, 4, 8]. Baggot et al. [2] reported that the proportion of digitoxin bound to albumin decreased in dogs after bilateral nephrectomy. Storstein [24] reported similar conditions in nephrotic human subjects. A small decrease in drug binding can significantly elevate the concentration of free drug that is active on the heart. Changing the digitoxin binding to plasma albumin and tissue protein can influence the distribution and the elimination rate of the drug, because the unbound free drug reaches the tissue or the site of drug action and is also metabolized and eliminated more readily.

Another important role of the canine kidney in digitoxin metabolism and excretion may be indicated by the fact that the radioactivity in the heart and liver was higher in the L group than the C group, and the radioactivity in the kidney was higher in the C group. These experiments have demonstrated the importance of the kidney in excreting digitoxin, just as Marcus et al. [14] found with digoxin.

Hamlin [8] reported that the plasma half-life of digitoxin is unaffected by nephritis and suggested that the kinetic behavior of digitoxin in the dog may be less susceptible to interference from extraneous factors. However, it appears that some factors may contribute to digitoxin metabolism and excretion, as described here. It has been predicted that the highly protein-bound and lipid-soluble digitoxin may undergo more complicated pharmacokinetics in the body as a result of many other pathological conditions influencing plasma protein concentration and drug disposition and the resulting renal protein loss (e.g. nephrotic syndrome).

In the present study of ³H-digitoxin turnover under the conditions of single intravenous injections in dogs with experimental acute renal failure, the plasma and tissue radioactivity increased and the plasma elimination half-life of the drug prolonged. This suggests that the kidney affects digitoxin metabolism and excretion, and is one of the more important kinetic determinants for the drug. Since it is thought that continued administration of digitoxin in usual doses in dogs with acute renal failure or other renal diseases may
result in digitalis intoxication caused by high plasma and tissue concentrations, the dosage or dose interval should be carefully adjusted with regard not only to liver function but also to renal function for patients with renal diseases who require digitoxin.

Further studies of digitoxin pharmacokinetics during maintenance therapy may still be required with normal dogs compared with those in various stages of pathological conditions, since digitoxin biotransformation and excretion in dogs is complex and different from that in human beings.

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REFERENCES

504–505.

要　約

実験的急性腎不全犬における血漿および組織内 3H-digitoxin 濃度：宮澤良道（帝京大学医学部第二内科学教室）—対照群（C 群）、両側尿管結石群（L 群）、両側腎摘出群（N 群）の実験犬に 3H-digitoxin を静脈内に１回投与し、その代謝・排泄に腎機能のどのように関与するかを digitoxin の血漿動態および組織内濃度より検討した。血漿 radioactivity は dichloromethane （CH2 Cl2）一溶性および一不溶性分画とも C 群にくらべ L および N 群で有意に高値を示した。CH2 Cl2 溶性分画の血漿排出率は C 群では 9.3 時間であったが、L、N 群ではその約 2 倍に延長し、digitoxin および強心作用を有する脂溶性代謝産物の血漿からの消失は著しく遅延した。Digitoxin 投与 24 時間後的心臓と肝臓の radioactivity は各群においてほぼ同じ濃度を示し、3 群間の比較では C、L、N 群の順に両組織の radioactivity が高くなり、有意差が認められた。一方、腎機能の radioactivity は心臓・肝臓よりも高値を示したが、両組織と異なり、C 群では L 群より高い傾向が認められた。各組織の radioactivity と血漿 CH2 Cl2 溶性分画の radioactivity との間には有意の正相関が存在した。これらの所見から、犬における digitoxin の体内動態において、腎機能が明らかに関与することが示唆された。