AN ALTERED DISTRIBUTION AND ELIMINATION OF DIGOXIN IN ANEMIC PATIENTS AND EXPERIMENTALLY INDUCED ANEMIC RATS

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Digoxin was administered orally to eight anemic patients in their anemic and convalescent stages, and serum digoxin concentration was determined by radioimmunoassay. In the anemic patients a significantly lower level of serum digoxin concentration was observed in anemic state compared with convalescent stage at 72 hours after the drug administration (p < 0.01). In usual clinical use, a full digoxin effect is expected to be attained as 72 hours. Tritiated digoxin was administered intravenously to anemic and control rats and the tritium in samples of the blood, myocardium and urine were counted in a liquid scintillation counter. The anemic rats showed significantly lower level of serum $^3$H-digoxin at 6 hours (p < 0.01) and lower myocardial concentration at 24 hours (p < 0.01). Larger amount of urinary excretion of $^3$H-digoxin was observed in the anemic rats 6 hours after the drug administration. No significant difference in fecal excretion of $^3$H-digoxin was found between the anemic and control rats.

Despite recent advances in knowledge of the chemistry and pharmacology of digitalis glycosides, digitalis intoxication is still a common clinical problem. The reported incidence of digitalis intoxication has ranged from 8 to 20 percent. Among the factors which influence digitalis sensitivity and resistance, anemia is rarely mentioned in the literature. We reported a case of digoxin intoxication which occurred in the convalescent stage of anemia with no factors except anemia to account for the intoxication. We suggested that metabolism of digoxin might be altered in anemic state.

In order to clarify the effect of anemia on the metabolism of digoxin, we employed both anemic patients and experimentally induced anemic rats for this study.

METHODS

Patients
After informed consent was obtained, 8 anemic patients participated in the study. One of the 8 patients had pernicious anemia and the rest had iron deficiency anemia. The mean hematocrit was 22 ± 3.4%. Digoxin was administered after an overnight fasting on the following dosage schedule: 1.5 mg on the first day and 0.5 mg on the 2nd and 3rd day, respectively. Patients were given digoxin tablets of the same brand and bottle. Blood samples were drawn in the fasted state at 2, 6, 24 and 72 hours after the first administration of digoxin and serum levels were determined by radioimmunoassay method.

Key Words:
Experimentally-induced anemia
Digoxin radioimmunoassay
Tritiated digoxin

(Received August 24, 1983; accepted June 9, 1984)
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TABLE I LABORATORY FINDINGS OF ANEMIC AND CONTROL RATS

<table>
<thead>
<tr>
<th></th>
<th>Anemic*</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>178.5 ± 6.7</td>
<td>185.0 ± 7.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>18 ± 2.3</td>
<td>42 ± 1.9</td>
</tr>
<tr>
<td>S-Na (mEq/L)</td>
<td>144 ± 4.5</td>
<td>142 ± 5.1</td>
</tr>
<tr>
<td>S-K (mEq/L)</td>
<td>6.1 ± 1.1</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>Urea N (mg/dl)</td>
<td>17 ± 1.8</td>
<td>18 ± 1.4</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.6 ± 0.12</td>
<td>0.7 ± 0.14</td>
</tr>
<tr>
<td>S-total protein (g/dl)</td>
<td>4.5 ± 0.4</td>
<td>4.6 ± 0.3</td>
</tr>
</tbody>
</table>

* N = 10  Mean ± S.D.

TABLE II LABORATORY FINDINGS OF 8 ANEMIC PATIENTS IN ANEMIC AND CONVALESCENT STAGES

<table>
<thead>
<tr>
<th></th>
<th>Anemic stage</th>
<th>Convalescent stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>22 ± 3.4</td>
<td>38 ± 4.3</td>
</tr>
<tr>
<td>S-Na (mEq/L)</td>
<td>141 ± 4.3</td>
<td>143 ± 3.6</td>
</tr>
<tr>
<td>S-K (mEq/L)</td>
<td>4.1 ± 2.7</td>
<td>3.8 ± 1.8</td>
</tr>
<tr>
<td>Urea N (mg/dl)</td>
<td>17 ± 1.8</td>
<td>18 ± 2.6</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.2 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td>S-total protein (g/dl)</td>
<td>7.2 ± 0.4</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>T4-RIA (µg/dl)</td>
<td>6.8 ± 1.3</td>
<td>7.4 ± 1.6</td>
</tr>
<tr>
<td>T3-RIA (µg/dl)</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

m ± S.D.

Treatment of Animals
Forty male Wistar strain rats 3 weeks of age and weighing 60–70 g, were used in the study. The experimental group of twenty were fed iron deficient feed and water for 4 weeks. They were bled drop by drop by cutting the end of the tail until the 22 ± 1.5% of hematocrit was attained. They were subsequently kept on the same feed for one more week when their hematocrit declined to 18 ± 2.3% and they weighed 178.5 ± 6.7 g. Iron deficient feed was provided by Oriental Kohbo Company. Twenty, control rats were maintained on common feed and running water. Body weight and laboratory data of anemic and control rats are shown in Table I.

Administration of 3H-digoxin
Digoxin, randomly tritium labeled (specific activity 16 Ci/mmol) was obtained from New England Nuclear Corp. Radiochemical purity of the 3H-digoxin was 98% tested by thin layer chromatography. 3H-digoxin in 1 ml of absolute ethanol was diluted with 10 ml of 0.9% saline solution and used in animal experiments within two months when the rate of decomposition is less than 1%. 3H-digoxin of 5 µg/100 g body weight was administered intravenously to the anemic and control rats and blood samples were obtained when they were decapitated 6 and 24 hours after the drug administration. Ten anemic and control rats were employed for the each time point. The rats were kept in metabolic cage throughout the experiment. Urinary samples were obtained at 6 and 24 hours and fecal samples at 24 hours after the drug administration.

Counting of 3H-digoxin
Serum and urine samples were extracted and subjected to thin layer chromatography according to the method described by Kolenda et al. It was established that nearly 90% of the radioactivity in the samples was digoxin. The serum and urine samples with 3H-digoxin, 0.5 ml each, were placed in counting vials containing 15 ml of the liquid scintillation medium reported by Bray. After 24 hours they were counted in a Packard Tri-Carb liquid scintillation counter. Serum and urine samples of the same quantity without 3H-digoxin were employed as background samples. Myocardial samples were minced in small pieces and dried. About 500 mg wet weight of the samples were burnt, extracted in the liquid scintillator by sample oxidizer (Packard model 306) and counted in the scintillation counter. Myocardial samples without 3H-digoxin were similarly treated and subjected to background samples. Fecal samples collected were treated and counted in the same method as myocardial samples.

Statistical analysis was made by Student's t test.

RESULTS

Table II shows laboratory findings of the 8

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anemic patients in their anemic and convalescent stages. The mean hematocrit was 22% in anemic stage while 38% in convalescent stage. Other laboratory findings including thyroid function tests were all normal in both anemic and convalescent stages.

Figure 1 demonstrates serum digoxin concentrations (determined by radioimmunoassay) of the 8 patients in anemic and convalescent stages. There is no significant difference in the serum concentrations between the anemic and the convalescent stages at 2 hours. This suggests an equivalent absorption capacity of digoxin in both stages. Significantly lower levels of serum digoxin concentrations are observed in anemic stage at 6 (p < 0.05) and 24 hours (p < 0.05) after administration. An even more significant level is detected at 72 hours (p < 0.01), when nearly full digoxin dose in clinical use is expected to be attained.

In order to analyze the mechanism of significant difference in the serum digoxin concentration in anemic and convalescent stages of human subjects, comparison study was made employing experimentally-induced anemic rats.

Table I summarizes laboratory findings of the experimentally induced anemic and control rats. The mean hematocrit level of anemic rats was 18% and control rats was 42%. Other laboratory findings showed no significant differences between the two groups.

Figure 2 demonstrates digoxin concentration expressed as $^3$H-counts in serum and myocardium of anemic and control rats at the indicated times after its administration. The anemic rats show significantly lower level of serum concentration at 6 hours (p < 0.01) and 24 hours (p < 0.05). Significantly lower level is observed in myocardial concentration of anemic rats both at 6 hours (p < 0.05) and 24 hours (p < 0.01).

Table III shows urinary excretion of $^3$H-digoxin in anemic and control rats. The amount excreted in the urine is expressed in total tritium counts and % excretion (% excretion versus administration of $^3$H-digoxin). The anemic rats show significantly larger urinary excretion of $^3$H-digoxin in 6 hours after its administration both in total counts (p < 0.01) and % excretion (p <
TABLE III  URINARY EXCRETION OF \(^3\)H-DIGOXIN IN 6 AND 7 TO 24 HOURS

<table>
<thead>
<tr>
<th></th>
<th>% Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm excreted</td>
</tr>
<tr>
<td><strong>6 hours</strong></td>
<td></td>
</tr>
<tr>
<td>Anemic 109,10 ± 13,200</td>
<td>16.0 ± 2.3</td>
</tr>
<tr>
<td>Controls 88,400 ± 13,500</td>
<td>11.7 ± 2.2</td>
</tr>
<tr>
<td><strong>7-24 hours</strong></td>
<td></td>
</tr>
<tr>
<td>Anemic 58,500 ± 14,800</td>
<td>8.5 ± 2.0</td>
</tr>
<tr>
<td>Controls 55,400 ± 13,100</td>
<td>7.5 ± 1.6</td>
</tr>
</tbody>
</table>

* = p < 0.01; ** = p < 0.001; N.S. not significant
N = 10

TABLE IV  FECAL EXCRETION OF \(^3\)H-DIGOXIN IN 24 HOURS

<table>
<thead>
<tr>
<th></th>
<th>% Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm excreted</td>
</tr>
<tr>
<td><strong>24 hours</strong></td>
<td></td>
</tr>
<tr>
<td>Anemic 19,100 ± 12,800</td>
<td>2.6 ± 1.8</td>
</tr>
<tr>
<td>Controls 16,900 ± 13,900</td>
<td>2.3 ± 2.3</td>
</tr>
</tbody>
</table>

N.S. = not significant

0.001). There is no statistically significant difference in 7 to 24 hours after administration.

Table IV shows amount of fecal excretion of \(^3\)H-digoxin in anemic and control rats in 24 hours expressed in both total counts and percentage of excretion. No significant difference in fecal excretion of \(^3\)H-digoxin is found between the two groups in either measurement.

DISCUSSION

As shown Fig. 1 significantly lower levels of serum digoxin concentrations are observed in the anemic stage at 6 and 24 hours (p < 0.05) after administration, and statistically more significant difference was detected between the two stages at 72 hours (p < 0.01) brought about by the additive effect of the repeated digoxin administration of 0.5 mg on the second and third day. The results observed in the 8 anemic patients in this study lend support to the previous implication that convalescence from anemia causes an increase in serum digoxin concentration which may result in digitalis intoxication.

Before discussing the pharmacokinetics of digoxin, it is of interest to compare it with digitoxin. More than 90% of digitoxin in the blood is bound to serum protein while less than 10% of digoxin is in bound form. The difference in the amount of protein binding in the serum is considered to contribute to the higher serum digitoxin levels (10–50 ng/ml) compared with digoxin (0.5–3 ng/ml). A study by Rieger et al. established the effect of protein binding on the uptake of ouabain and digitoxin into the heart. Rasmussen et al. found that serum digitoxin level was directly related to the plasma protein level. These facts suggest the relationship of serum protein binding in the tissue distribution and retention of digitoxin.

On the other hand, less than 10% of digoxin in the blood is bound to serum protein and more than 50% resides in the water soluble fraction of erythrocytes. Only a few percent of digitoxin is recoverable from them. It has been proposed that digoxin in the erythrocytes plays an important part in its tissue distribution, retention and excretion. To investigate this possibility, an animal model was established. Experimental rats were utilized because the erythrocytes of rats contain the highest amount of digoxin of all mammals, including man. Therefore they serve to typify the role of erythrocytes in the pharmacokinetics of digoxin. Anemia was produced at a greater rate in the experimental rats than in human subjects. The hematocrit in rats was 18% versus 23% in human subjects. As anemic rats on continued iron deficient feed lost significant weight as a result of hypoproteinemia, the acute bleeding method was utilized. In order to induce more prominent anemia with less hypoproteinemia, the three-step method was developed: iron deficient feed, bleeding by the above mentioned method, and maintenance on the iron deficient feed.

Figure 2 demonstrates that both myocardial and serum digoxin concentrations were significantly lower in anemic rats than in controls. As shown in Table III, the anemic rats produced significantly more urinary excretion of digoxin in 6 hours after administration. This may account for the lower serum and myocardial concentration in anemic rats compared with controls.

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Fig. 3. A proposal scheme for elimination of digoxin with intravenous injection.

Similar to many other drugs, digoxin follows the two compartment open model after intravenous administration. According to the model, the serum turnover curve after a single intravenous bolus of a drug, is composed of two distinct linear components. The initial rapid fall in concentration is the distribution phase where in the central and peripheral compartments make equilibration. The latter component is the elimination phase in which the drug disappearance is determined mainly by irreversible elimination from the central compartment. In view of the results of urinary excretion, and the serum and myocardial digoxin concentration in the experimentally induced anemic rats in this study, the following schemes may be proposed to clarify the possible pharmacokinetics of digoxin in anemic state. Hinderling et al. described in their study on the protein binding and erythrocyte partitioning of disopyramide that erythrocyte may be considered to be a “subcompartment” of the central compartment. Fig. 3 shows our hypothetical scheme of erythrocytes as subcompartment of the central compartment in the pharmacokinetics of digoxin. In the distribution phase, erythrocytes as subcompartment make equilibration with serum compartment and the serum compartment subsequently equilibrates with peripheral compartment. Since the erythrocyte mass as a subcompartment is smaller in quantity in the anemic state, its capacity to preserve digoxin is decreased. This leaves a larger amount of digoxin in the serum which is available for increased urinary excretion in 6 hours (Table III). The amount of digoxin distributed to peripheral compartment is decreased, which results in a lower myocardial digoxin concentration in the anemic state. In the distribution and in the elimination phase, a decreased capacity of erythrocytes to preserve digoxin brings about increased elimination of digoxin from peripheral compartment. This results in lower myocardial digoxin concentration in the anemic state. The net effect of the equilibration between central and peripheral compartment in the distribution and elimination phase contributes to the distinctly lower myocardial digoxin level (p < 0.01) 24 hours after its administration (Fig. 2).

A question may be raised that as most of the body load of digoxin is tissue bound, this would constitute a much larger reservoir than that in erythrocytes influencing serum digoxin concentration. Although tissue/serum ratio of digoxin concentration is a several to fifty in various tissues, most of the digoxin in tissue is thought to be firmly bound to tissue proteins. The smaller amount of unbound form of digoxin in tissue is available for dynamic equilibration with serum digoxin. Therefore, as more than 50% of digoxin in the blood is in the erythrocytes, it is quite conceivable that erythrocyte digoxin bears as much influence on serum digoxin concentration as tissue digoxin.

Another question may be raised that the decreased serum and myocardial digoxin concentration in the anemic state may be attributed to increased serum volume and increased intramyocardial water volume. But the same inclination in chronic anemia was observed in the acute anemic state as in our experimentally induced anemia. In this case the serum volume was not thought to be increased.

Analogous pharmacokinetic studies are reported on disopyramide and thiamine propyl disulfide. A proper chemical modification of thiamine brings about an increase in tissue retention. Nogami and colleagues established that thiamine propyl disulfide is absorbed into erythrocytes, which attributes to the longer half-life of the thiamine derivatives. These reports on disopyramide and thiamine propyl disulfide support our hypothesis: Erythrocytes may play an important role in the distribution and elimination of drugs which are considerably absorbed into erythrocytes. The results of the present study support the conclusion that serum and myocardial digoxin levels are decreased in the anemic state and increased in the convalescent stage. This results in digoxin intoxication when the same maintenance dose is continued. The clinical implication that may be concluded is that in the anemic state a higher amount of digoxin maintenance dose is necessary, and in the convalescent stage it should be curtailed.

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