 Interruption of the Enterohepatic Circulation of Digitoxin by Cholestyramine

II. EFFECT ON METABOLIC DISPOSITION OF TRITIUM-LABELED DIGITOXIN AND CARDIAC SYSTOLIC INTERVALS IN MAN

JAMES H. CALDWELL, CHARLES A. BUSH, AND NORTON J. GREENBERGER

From the Divisions of Gastroenterology and Cardiology, Department of Medicine, The Ohio State University College of Medicine, Columbus, Ohio 43210

ABSTRACT Previous studies of digitalis glycoside metabolism and excretion have indicated that these compounds undergo a significant enterohepatic cycle in some species. It has been suggested that the existence of such a cycle in man contributes to the prolonged action of certain cardiac glycosides. Previous studies have demonstrated that cholestyramine binds digitoxin and digoxin in vitro and accelerates the metabolic disposition of digitoxin in rats and guinea pigs, presumably by interrupting the enterohepatic circulation.

In order to assess the role of the enterohepatic circulation in the metabolism of digitalis glycosides in humans, maintenance doses of cholestyramine were administered to 7 of 15 normal human subjects beginning 8 hr after digitalization with 1.2 mg of digitoxin-14C. All subjects had frequent measurements of serum radioactivity, left ventricular ejection time (LVET), and electromechanical systole (Qs), the latter recorded as the interval from onset of Q wave to first major component of second heart sound. Measurement of the LVET and QS intervals affords a sensitive index of the cardiac response to digitalis. In addition, chloroform extraction of serum was performed to separate unchanged digitoxin and active metabolites from cardiovascular metabolites of digitoxin. Cholestyramine treatment resulted in reduction in half-life to total serum radioactivity from 11.5 to 6.6 days, and in chloroform-extractable radioactivity from 6.0 to 4.5 days, as compared to controls. In addition, cholestyramine treatment was accompanied by more rapid return to baseline levels of digitoxin-induced changes in the LVET and QS intervals. A significant positive correlation was found between QS values and chloroform-extractable radioactivity, the latter reflecting unchanged digitoxin-14C (r=0.64; P<0.01).

The results indicate that administration of cholestyramine to digitalized human subjects accelerates the metabolic disposition of digitoxin and abbreviates the physiologic response to the glycoside. This effect is presumably mediated by interruption of the enterohepatic circulation of digitoxin by cholestyramine.

INTRODUCTION

Recent studies from this laboratory (1) have demonstrated that cholestyramine, an anion exchange resin, binds substantial amounts of the cardiac glycosides digitoxin and digoxin in vitro. Further, in rats and guinea pigs, it was shown that pretreatment with cholestyramine provides appreciable protection against lethal doses of digitoxin (1). This effect, accompanied by accelerated fecal excretion and reduced levels of digitoxin in some tissues, is apparently mediated by interruption of the enterohepatic circulation of digitoxin. Although the original concept of the enterohepatic cycle of cardiac glycosides was proposed by Okita, Talso, Curry, Smith, and Geiling on the basis of early studies on the metabolism of these drugs in humans (2), the relevance of this enterohepatic circulation to clinical pharmacology has not been fully appreciated (3). This report describes studies carried out to evaluate the effects of pharmacologic interruption of the enterohepatic circulation of digitoxin in man by cholestyramine. The data obtained indicate that cholestyramine treatment causes a significant shortening of the metabolic half-life of circulating digitoxin-14C and enhanced dissipation of its cardiac effects as determined by serial changes in cardiac systolic intervals.

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METHODS

Materials. Tritium-labeled digitoxin1 (SA 1.0 mCi/0.133 mg) was checked for purity by thin-layer chromatography and found to be >98% pure. Commercial digitoxin was obtained as Purodigin2 in ampules containing 0.2 mg/ml in 40% alcohol. Cholestryamine was kindly supplied as a gift.3

Measurement of radioactivity. Blood was obtained from subjects by venipuncture and serum separated by centrifugation. Duplicate 0.5 ml portions of serum were added to 15.0 ml of Bray’s solution (120 g naphthalene, 8 g POPOP (2,5-diphenyloxazole), 400 mg POPOP (1,4-bis[2-(3-phenylloxazoyl)]-benzo), 200 ml absolute methanol, 40 ml ethylene glycol brought to a final volume of 2000 ml with p-dioxane) in polyethylene counting vials. Samples were counted in a Packard Tri-Carb scintillation spectrometer model 33754 equipped with automatic external standardization. Counting efficiency was 20%. Enough counts were taken on each sample to assure a counting error of less than 3%.

Chloroform extraction of serum samples was carried out by a modification of the method of Katzung and Meyers4. This method separates unchanged digitoxin-8H and its chloroform-soluble metabolites from the water-soluble metabolites. The major chloroform-soluble metabolite of digitoxin is digoxin (3, 5). The intermediary metabolites of digitoxin and digoxin have poorly established biologic activity and cannot usually be detected in vivo (6), as they are rapidly converted to water-soluble compounds with little or no biologic activity (6, 7). Chloroform extraction thus separates the labeled glycossides of the serum into a chloroform-soluble fraction containing primarily digitoxin and digoxin, and a water-soluble fraction containing degradation products of these substances. Duplicate 0.5 ml portions of serum were extracted with 1.5 ml chloroform, the aqueous phase removed by pipette and extracted twice more with 1.0 ml chloroform, and the chloroform phases pooled and rinsed into polyethylene counting vials with additional chloroform. The chloroform phase was allowed to evaporate in a hood at room temperature and 15.0 ml Bray’s solution added to the radioactive residue and shaken. In preliminary experiments, 93–99% of digitoxin-8H added to control serum was recovered by this procedure.

Regression analysis of data on total and chloroform-extractable serum radioactivity was done by transformation of variables and linear regression using a modification of a Wang program (Wang Laboratories,4 CAL 360-STAT 6) and a Hewlett-Packard 9100 A calculator.5 Measurement of systolic intervals. Duration of the phases of electrical and mechanical systole was determined by the method of Weisler, Snyder, Schoenfield, and Cohen (8). All subjects rested for at least 30 min in a quiet room and abstained from food, tobacco, or caffeine for 4 hr before each systolic interval determination. Simultaneous recordings of the electrocardiogram, phonocardiogram, and carotid arterial pulse were made on a multichannel recorder at a paper speed of 100 mm/sec with 20 msec time lines. The phonocardiogram was recorded from a Feiker microphone placed in a constant position on the chest for each recording, at a point where clear inscription of both heart sounds could be detected. The carotid pulse tracing employed a funnel-shaped pickup connected to an air-filled Statham P23D6 transducer,6 placed over the point of maximal pulsation of the carotid artery.

The phases of systole determined from these measurements included (a) the Q&S interval,7 which is defined as the interval from the onset of the Q wave to the first major component of the second heart sound and (b) the left ventricular ejection time (LVET), which is the interval from the upstroke to the incisura of the carotid arterial pulse tracing. Both intervals were corrected for heart rate (HR) using the regression equations derived from data previously obtained for normal male subjects (9):

\[ Q+S = -2.1\ HR + 546, \]
\[ LVET = -1.7\ HR + 413. \]

The corrected LVET is known as the ejection time index (ETI). Deviations from the normal in QS and LVET intervals were calculated as the difference between the observed interval and that predicted from the normal regression equation (AQ&S, ALVET). Corrections for diurnal variation in intervals recorded at 8 hr after ingestion of digitoxin was made by subtracting the mean 8 hr reduction in intervals measured in normal subjects from the observed 8 hr measurement (9). Statistical analyses were performed by use of the Student t test (10).

Experimental design. 15 healthy male medical students and physicians ranging in age from 21 to 35 served as volunteer subjects. All subjects were found to be normal on physical examination and had normal electrocardiograms. Subjects were divided at random into control and cholestramine treatment groups before participation. On the morning of the first study day, the fasting subjects rested in a quiet room and had replicate base line measurements of systolic intervals performed. The subjects then ingested 1.2 mg of digitoxin-8H (100 μCi) in 40% ethyl alcohol. Serial blood samples were taken at 30, 60, and 90 min, 2, 4, 6, and 8 hr after ingestion of digitoxin-8H and at 24 hr intervals for 1 wk thereafter. Systolic intervals were recorded 4 and 8 hr after ingestion and at 24 hr intervals thereafter for 1 wk. In addition, the cholestyramine-treated subjects took 4 g cholestyramine 8, 12, and 16 hr after the dose of digitoxin-8H and four times daily thereafter for 5 days. Two control subjects noted nausea for a few minutes within 1 hr after ingestion of digitoxin-8H. Three cholestyramine-treated subjects noted slight constipation and one experienced nausea following the medication for the first 2 days. No subject considered the symptoms severe enough to warrant discontinuation of the study. No arrhythmias were recorded in either group during the study.

RESULTS

Effect of cholestyramine on blood levels of digitoxin-8H. Serum levels of total radioactivity after ingestion of 1.2 mg of digitoxin-8H in control and cholestyramine-treated subjects are shown in Fig. 1. It is apparent that maximal levels of radioactivity were

2 Wyeth Laboratories, Philadelphia, Pa.
3 Mead Johnson & Co., Evansville, Ind.
4 Packard Instrument Co., Inc., Downers Grove, Ill.
5 Wang Laboratories, Tewksbury, Mass.
6 Hewlett-Packard Co., Palo Alto, Calif.
7 Statham Instruments, Inc., Los Angeles, Calif.
8 Abbreviations used in this paper: ETI, ejection time index; HR, heart rate; LVET, left ventricular ejection time; QS interval, interval from onset of Q wave to first major component of second heart sound.
reached within 60–90 min after ingestion and that blood levels of digitoxin-3H were essentially similar through the first 8 hr, at which time the treatment group began taking cholestyramine. Thereafter the serum radioactivity fell more rapidly in the cholestyramine-treated subjects, and was significantly lower at 48 hr and at each time interval thereafter until the end of the study. It is important to note that serum radioactivity 8 hr after ingestion of digitoxin-3H averaged 5006 dpm/ml in control subjects and 5078 dpm/ml in subjects taking cholestyramine.

**Figure 1** Effect of cholestyramine treatment on total serum radioactivity after ingestion of 1.2 mg (100 μCi) of digitoxin-3H. The subjects received 16 g of cholestyramine daily for 7 days commencing 8 hr after ingestion of digitoxin. The values shown represent the mean ±1 sd.

**Figure 2** Effect of cholestyramine on the half-life (t½) of total serum radioactivity after ingestion of 1.2 mg (100 μCi) of digitoxin-3H. The values shown are derived from the data in Table I.

**Figure 3** Effect of cholestyramine on the half-life (t½) of serum chloroform-extractable radioactivity after ingestion of 1.2 mg (100 μCi) of digitoxin-3H. The values shown are derived from the data in Table II.
### Table 1

**Total Serum Radioactivity after Oral Administration of Digitoxin-3H***

<table>
<thead>
<tr>
<th>Group</th>
<th>Hr after ingestion of digitoxin-3H</th>
<th>Serum radioactivity (dpm/ml)</th>
<th>t1/2 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control Subjects</td>
<td>8</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>1</td>
<td>4794</td>
<td>3978</td>
<td>3900</td>
</tr>
<tr>
<td>2</td>
<td>5384</td>
<td>4806</td>
<td>4672</td>
</tr>
<tr>
<td>3</td>
<td>4318</td>
<td>3918</td>
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<tr>
<td>4</td>
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<td>4746</td>
</tr>
<tr>
<td>8</td>
<td>3790</td>
<td>4330</td>
<td>3918</td>
</tr>
<tr>
<td>Mean</td>
<td>5006</td>
<td>4524</td>
<td>4467</td>
</tr>
</tbody>
</table>

2. Cholestyramine Subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Hr after ingestion of digitoxin-3H</th>
<th>Serum radioactivity (dpm/ml)</th>
<th>t1/2 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4539</td>
<td>4476</td>
<td>4520</td>
</tr>
<tr>
<td>2</td>
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<tr>
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<td>4772</td>
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<td>3362</td>
</tr>
<tr>
<td>Mean</td>
<td>5078</td>
<td>4743</td>
<td>4027</td>
</tr>
</tbody>
</table>

* Subjects received 1.2 mg of digitoxin-3H (100 µCi) in 40% ethyl alcohol. For further experimental details see text.

† t1/2 was calculated by regression analysis done by transformation of variables and linear regression using a modification of a Wang Program (Wang Laboratories, CAL 360-STAT 6).

‡ Mean ±1 SD.

tyramine. Since the specific activity of the ingested glycoside was 1.2 mg/100 µCi or 12 ng/2260 dpm, it was calculated that mean peripheral blood digitoxin-3H levels were 26.5 and 26.8 ng/ml in the two groups respectively, well within the range of values reported by others (11, 12) after a comparable dose of digitoxin.

Regression analysis of all the individual data points from which the mean values in Fig. 1 were derived was carried out for each individual subject using the formula $y = ae^{-t}$ (Table I). The mean t1/2 for the slow linear process of decline of serum radioactivity was calculated to be $11.5 \pm 2.3$ (±1 SD) days in the control subjects and 6.6 ±1.9 days in the cholestyramine-treated subjects ($t = 4.2; P < 0.01$). This reflects a substantial difference in the rate of metabolic clearance and excretion of digitoxin and metabolites from the blood in these two groups. Fig. 2 shows a semilogarithmic plot of the data in Table I and Fig. 1. Extrapolation of these two curves to zero time, and subtracting from the raw curve, permits one to draw a line representing the early phase of tissue distribution of the glycoside. This plot was virtually identical in both groups, the t1/2 being 2–3 hr. These observations indicate that cholestyramine, given in the usual maintenance doses, caused a significant reduction in the half-life of total radioactivity-3H in acutely digitoxinized human subjects. As indicated above, total radioactivity-3H includes unchanged digitoxin, as well as cardioactive and inactive metabolites.

It will be recalled that chloroform extraction separates the labeled glycosides into a chloroform phase representing predominantly cardioactive compounds, including all of the unchanged digitoxin, and an aqueous phase containing essentially cardioactive but radiolabeled metabolites. To determine whether cholestyramine caused a shortening of the half-life of cardioactive radioactivity, chloroform-extractable radioactivity in the serum was measured (Table II). The mean t1/2 of chloroform-soluble radioactivity in the control group was calculated to be $6.0 \pm 0.9$ days, approximating the physiological half-life reported by other investigators (7, 8). By contrast, the half-life of digitoxin-3H in the cholestyramine-treated group was significantly reduced to $4.5 \pm 0.9$ days ($t = 3.1; P < 0.01$). Fig. 3 shows a semilogarithmic plot of the data in Table II. The data clearly indicate that the reduction in total radioactivity seen in the cholestyramine-treated subjects was paralleled by a similar decrease in the level of cardioactive glycoside radioactivity, predominantly unchanged digitoxin.

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Thereafter, the mean 1 of cholestyramine-treated subjects following ingestion is virtually identical to that of normal subjects. These values for QS2 at 8 hr are very similar to those previously reported following oral ingestion of 1.6 mg digitoxin (15 ± 2.1 msec) (8). Thereafter, the values in the cholestyramine-treated subjects return toward normal much more rapidly than those in the control subjects.

Effect of cholestyramine on systolic intervals. Data on the ejection time index from 8 hr to 5 days after ingestion of 1.2 mg digitoxin by control and cholestyramine-treated subjects is summarized in Table III. It can be seen that at 24 hr both groups of subjects had virtually identical reduction in LVET from control levels (14.5 ± 1.7 vs. 14.5 ± 2.3 msec). These values at 24 hr are very similar to that previously reported following oral ingestion of 1.6 mg digitoxin (15 ± 2.1 msec) (8). Thereafter, the values in the cholestyramine-treated subjects return toward normal much more rapidly than those in the control subjects.

Weissler and Schoenfeld have recently presented evidence that the QS2 interval is a more constant and specific index of cardiac digitalis effect (9). The effect of cholestyramine on the digitoxin-induced changes in the QS2 interval in the same groups of subjects is seen in Table III and Fig. 4. It can be seen that while values for ΔQS2 are similar at 8 hr (19.6 ± 3.0 vs. 20.3 ± 2.7 msec), the further decrease in ΔQS2 values is accelerated in the subjects taking cholestyramine. The values in the cholestyramine-treated subjects are significantly different from those in the control subjects 4 and 5 days after digitalization. These data indicate

![Figure 4](http://www.jci.org)  
**Figure 4** Effect of cholestyramine treatment on the QS2 systolic interval following ingestion of 1.2 mg digitoxin. The subjects received cholestyramine as described in the text and Fig. 1.
that cholestyramine accelerates the dissipation of contractile responses to digitoxin in a manner similar to the reduction in peripheral blood levels of digitoxin-H.

To further corroborate the relationship between blood levels of digitoxin-H and physiologic response, individual values for simultaneously determined QSs and chloroform extractable radioactivity were compared by regression analysis (Fig. 5). It can be seen that there is a significant correlation between the serum radioactivity and the abbreviation of electromechanical systole due to digitoxin (r = 0.654, P < 0.01). The apparent wider dispersion of points at ΔQSs values less than 10 msec probably reflects the relatively greater difficulty in accurately measuring the small changes in QSs found in the cholestyramine-treated subjects in the latter part of the study. The data indicate that there is a positive correlation between myocardial electromechanical response and peripheral blood cardiac glycoside levels, the latter presumably reflecting myocardial digitoxin levels.

DISCUSSION

Recent studies concerned with the metabolism and clinical pharmacology of the cardiac glycosides have suggested that enterohepatic circulation of these drugs may influence the duration of action of a given compound (13). The studies reported herein were performed in order to determine whether pharmacologic interruption of this enterohepatic cycle was feasible in the acutely digitalized human subject. Determinations of total radioactivity and chloroform-soluble radioactivity in peripheral blood following the ingestion of digitoxin-H were carried out in control and cholestyramine-treated subjects. These data provide a means for determining whether oral administration of a binding agent such as cholestyramine would influence peripheral blood levels of cardioactive labeled glycoside in the digitalized human. In addition, measurement of systolic intervals in these subjects afforded a means of assessing the physiologic effects of digitoxin on the heart and permitted comparison between simultaneous blood level of digitoxin and cardiac responses. The systolic interval determinations have been shown to be a predictable and dose-dependent measurement of physiologic response of the heart to cardiac glycosides (8), and thus provide an additional parameter by which the effects of cholestyramine can be assessed.

The parallel reduction in digitoxin blood levels (Figs. 1-3) and contractile response to digitoxin (Fig. 4) in the cholestyramine-treated subjects clearly indicate that such treatment shortens the metabolic half-life and enhances the physiologic dissipation of the cardiac effects of digitoxin. There are several lines of evidence indicating that these effects are the result of interrup-

![Figure 5 Correlation between the QSs systolic interval and serum chloroform extractable radioactivity following ingestion of 1.2 mg (100 μCi) of digitoxin-H. Cholestyramine treatment was as described in the text and Fig. 1.](https://doi.org/10.1172/JCI106764)

**Table III**

*Effect of Cholestyramine on Digitoxin-Induced Changes in Electromechanical Systole (QSs) and Ejection Time Index (ETI)*

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Control (8)</th>
<th>Cholestyramine (7)</th>
<th>Control (8)</th>
<th>Cholestyramine (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>19.6 ±3.0†</td>
<td>20.3 ±2.7</td>
<td>18.2 ±2.5</td>
<td>14.4 ±3.9</td>
</tr>
<tr>
<td>1</td>
<td>26.0 ±2.1</td>
<td>18.9 ±2.9</td>
<td>14.5 ±1.7</td>
<td>14.5 ±2.3</td>
</tr>
<tr>
<td>2</td>
<td>19.3 ±1.3</td>
<td>17.7 ±3.6</td>
<td>15.1 ±1.7</td>
<td>11.7 ±3.2</td>
</tr>
<tr>
<td>3</td>
<td>17.8 ±1.7</td>
<td>15.3 ±2.4</td>
<td>12.5 ±0.8</td>
<td>12.2 ±1.9</td>
</tr>
<tr>
<td>4</td>
<td>17.3 ±1.1</td>
<td>10.9 ±1.8†</td>
<td>13.4 ±0.8</td>
<td>5.6 ±2.4†</td>
</tr>
<tr>
<td>5</td>
<td>15.3 ±0.6</td>
<td>7.1 ±2.5†</td>
<td>14.1 ±1.4</td>
<td>3.0 ±2.3†</td>
</tr>
</tbody>
</table>

* The QSs and ETI data are expressed as milliseconds difference from average of triplicate base line determinations. Numbers in parentheses refer to numbers of subjects.
† Mean ±1 sd.
‡ P < 0.05.
§ P < 0.01.
¶ P < 0.02.
Fourth, in the human studies described in this report, the 8 hr interval between administration of digitoxin and initiation of cholestyramine treatment allows for complete absorption, distribution, and tissue uptake of the glycoside to occur before the first dose of the resin. This would seem to exclude the possibility that the cholestyramine effects were due to interference with the initial absorption of the glycoside. Fifth, no changes in serum electrolytes that might alter cardiac responsiveness to digitoxin are known to occur following cholestyramine (1). Finally, there is no evidence that cholestyramine affects the hepatic metabolism of cardiac glycosides. Goldfinger, Heizer, and Smith (14) have recently presented evidence that cholestyramine administered concurrently with oral maintenance doses of digitoxin or digoxin results in lowered serum levels of the glycoside, presumably as a result of direct interference with absorption of the drug. Although the method used by these investigators does not allow estimation of the role of the enterohepatic circulation in human cardiac glycoside metabolism, some of their results are pertinent in regard to the present study. For example, they have performed in vitro binding studies that corroborate the results reported from this laboratory (1). They have also demonstrated that cholestyramine binds considerable amounts of digitoxin and digoxin in the intestinal lumen of man, resulting in reduced serum levels of these glycosides. In addition, they have demonstrated normal absorption of digoxin in one patient with complete biliary occlusion. This preliminary evidence that bile is not required for the normal intestinal absorption of digitalis confirms earlier animal studies from this laboratory (15). Furthermore, such data suggest that the direct binding of cardiac glycosides by cholestyramine is sufficient to account for the interference with glycoside absorption observed in this and other studies (1). It does not seem necessary to postulate that the cholestyramine-induced inhibition of glycoside absorption is mediated by the binding of bile salts.

The data obtained in this study provide indirect evidence for the existence of a significant enterohepatic cycle for digitoxin in humans. Furthermore, our observations suggest that the enterohepatic circulation of digitoxin contributes to the prolonged action characteristic of this glycoside. Finally, it was demonstrated that cholestyramine treatment caused a significant shortening of the metabolic half-life and enhanced physiologic dissipation of the cardiac effects of digitoxin, the latter measured by serial changes in systolic intervals. It is suggested that these effects were caused by intraluminal binding of digitoxin by cholestyramine with resultant interruption of the enterohepatic cycle of this glycoside. However, it remains to be established whether such pharmacologic interruption of the enterohepatic circulation of digitoxin will prove to be of value in the treatment of patients with digitalis intoxication.

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REFERENCES