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METABOLISM OF CARDIAC GLYCOSIDES—II

CARDIOTOXICITY OF METABOLITES OF DIGITOXIN-7αT*

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Abstract—The methodology of isolating and purifying large quantities of two highly polar metabolites of digotoxin-7αT by giving KCl concurrently to rabbits is described. The effect of concomitant KCl administration and the effect of large doses of digitoxin on the metabolism of the glycoside were evaluated. The cardiotoxicity of the isolated metabolites in cats has been determined. The data show that KCl does not qualitatively alter the metabolism of digitoxin in rabbits, although the toxicity of the glycoside at the dose employed is abolished. Evidence is also presented which indicates that the large doses of glycoside did not qualitatively alter the metabolism of the drug. When the two isolated metabolites of digitoxin were bioassayed in cats, it was found that they were at least three times more toxic than the parent compound. Thus it was concluded that digitoxin metabolism in the rabbit was unique since other species generally convert the digitoxin to less toxic compounds.

THE METABOLIC and excretory patterns of cardiac glycosides have been studied in a variety of animal species[1–3] as well as in healthy humans and in patients with cardiac decompensation or renal failure.[4–7] In an earlier report, we presented data that indicated severe heart failure in rabbits resulted in a reduced conversion of digitoxin to its more polar metabolites and an overall reduced rate of urinary excretion of the glycoside and its metabolites.[8]

The present study was designed to determine the ability of rabbits to detoxify digitoxin by comparing the cardiotoxicity of the two major isolated metabolites with the parent compound.

METHODS

Isolation of metabolites. Three separate batches of digitoxin-7αT were biosynthesized and isolated as previously described.[8] Carrier digitoxin (45 mg) was added to each batch of digitoxin-7αT, and each mixture was crystallized four times from aqueous ethanol until a constant specific activity for each batch was achieved. The specific activity of each batch was, respectively, 0·067, 0·072 and 0·047 mc/mg.

Three adult virgin albino rabbits (3·6 ± 0·31 kg) were given intramuscular injections of digitoxin-7αT. Each rabbit received a dose of 1·0 mg every 4 hr for 40

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doses. Intraperitoneal injections of KCl (400 mM/l.) at a dose of 4·5 mg/kg were given 4 hr prior to digitoxin administration and every 6 hr thereafter to prevent digitoxin toxicity. KCl administration was continued for 48 hr after the final injection of digitoxin, and each animal received approximately 570 mg of KCl over the total 212-hr experimental period.

The total amount of radioactivity given ranged from $4{\cdot}17 \times 10^6$ to $6{\cdot}39 \times 10^6$ dis./min (calculated pooled specific activity of 0·06 μc/mg), as shown in Table 1. A fourth rabbit (4·0 kg), which served as a control, received a single intramuscular injection of 0·88 mg (0·376 μc/mg) of digitoxin-7αT but did not receive KCl. The purpose of the control rabbit, which received a low dose of digitoxin and no KCl, was to determine whether a large dose of glycoside or the presence of KCl as received by the other rabbits altered the metabolism of the glycoside.

TABLE 1. DOSAGE AND ACTIVITY (DPM) OF DIGITOXIN-7αT RECEIVED OVER 7-day PERIOD BY EACH RABBIT

Rabbit no.	R_1	R_2	R_3	Control*
Total digitoxin-7αT received (mg)	40·0	40·0	40·0	0·88
Total activity received (μc)	2·68	2·88	1·88	0·331
Total DPM received	$5{\cdot}95 \times 10^6$	$6{\cdot}39 \times 10^6$	$4{\cdot}17 \times 10^6$	$7{\cdot}35 \times 10^5$

* Total amount given in a single injection.

Standard rabbit metabolic cages were utilized to prevent any mixing of urine and feces. Urine was collected daily for 24 days after the initial injection of digitoxin-7αT and stored at $-6°$. At the time of the final urine collection, the samples from each rabbit were thawed, individually pooled, filtered through glass wool, and reduced to dryness by lyophilization. The pooled urine from each rabbit was processed separately and distributed through 99 countercurrent transfers in ethyl acetate–cyclohexame–ethanol–water (7:3:3:7). Each fraction was reduced to dryness under nitrogen and redissolved in methanol (10 ml). For determination of tritium content, an aliquot (0·5 ml) was added to 5 ml of ethanol and 10 ml of a toluene solution of liquid scintillator. A correction for quenching was made by the use of tritiated toluene as an internal standard. K values were determined for the major peaks of activity from the test rabbits (R_1, R_2, R_3) and compared to the major peaks of activity obtained from the control rabbit. The two most polar peaks (Peaks A and B) from the countercurrent distributions of the test rabbits were also compared with the two respective peaks from the control rabbit by analysis in two different paper chromatographic systems: (1) chloroform–benzene–butanol (78:12:5) saturated with formamide, and (2) methylene chloride saturated with formamide. In both systems, the paper was saturated with a solution of 30 per cent formamide in acetone. Peak tubes from Peak A (R_1) were combined and evaporated to dryness and the residue was dissolved in methanol, placed on a thick-layer silica gel H (Merck) plate, and developed in chloroform–ethanol (7:3). The procedure facilitated a separation of a single radioactive zone from various pigments and salts in the mixture. The radioactive zone was scraped from the chromatographic plate. An identical procedure was undertaken with

the peak tubes from Peak A of R_2 and R_3. The three zones of radioactivity were combined, dissolved in ethanol, filtered and crystallized four times from aqueous ethanol until a constant specific activity of 0·06 μc/mg was obtained.

This entire procedure was repeated with each Peak B; again a constant specific activity of 0·06 μc/mg was obtained.

The radioactivity under each Peak C was pooled and submitted to thin-layer chromatography in a previously described system.

Bioassay of metabolites (Peaks A and B). The relative toxicities of Peaks A and B, digitoxin, and ouabain were determined by the cat assay procedure described by Chen and Chen.[9] Briefly, cats of either sex, weighing between 1·7 and 3·0 kg, were fasted for 16 hr and lightly anesthetized with ether. The right demoral vein was cannulated and prepared for infusion, and EKG leads were implanted for continuous monitoring and recording of electrocardiograms taken from lead II. Preliminary studies were done to estimate the concentrations of compounds that would allow the infusion rates to be identical, and the time until expiration (isoelectric EKG) with each compound to be 20–40 min, with the total infusion volume ranging from 15 to 25 ml. In each instance, prior to expiration the classic EKG changes, indicative of cardiac glycoside toxicity, were observed. These included slowed heart rate, prolongation of P-R interval, A-V dissociation, secondary tachycardia and ventricular fibrilation. The following concentrations were used: Peak A and Peak B were 1:72,000, digitoxin 1:34,000 and ouabain 1:68,000 or 1:72,000. All solutions were prepared fresh daily, and each was infused into four cats at the rate of 0·573 ml per min until cardiac arrest occurred. The quantity of compound required to produce cardiac arrest was calculated and recorded.

RESULTS

Isolation of metabolites. The rabbits tolerated the large doses of digitoxin-7αT with no overt toxicity. Preliminary experiments showed that rabbits that received an intramuscular injection of digitoxin of 1·2 mg/kg or greater, and no KCl, would convulse and die within 2 hr. The radiochromatograms obtained by countercurrent distribution of the urines from the four rabbits are shown in Fig. 1. The total urinary recovery of radioactivity for the three experimental rabbits, R_1, R_2, R_3, were, respectively, 60, 68 and 54 per cent; the recovery for the control rabbit was 62 per cent. These recoveries of radioactivity compare favorably with that previously reported by us.[8] The per cent distributions of the recovered radioactivity under the three major peaks of the countercurrent distribution are shown in Table 2. These data also compare favorably with those previously reported.[8] The similarities of the calculated K values (see Table 2) of the four A peaks and the four B peaks indicate that the large doses of digitoxin-7αT were metabolized in the same manner as a low dose. The four C peaks, although not confirmed by crystallization in this study, were separated by means of thin-layer chromatography as previously described.[8] Again, Peak C radioactivity was separated into three separate peaks that corresponded to the three cospotted standards, i.e. digitoxin, digitoxigenin-bis-digitoxoside, digitoxigenin-monodigitoxoside. No radioactivity was found that corresponded to the cospotted digoxin standard. A relatively high percent of Peak C in the test rabbits (43·4 $\pm$ 3·1) was observed as compared with control (20·1).

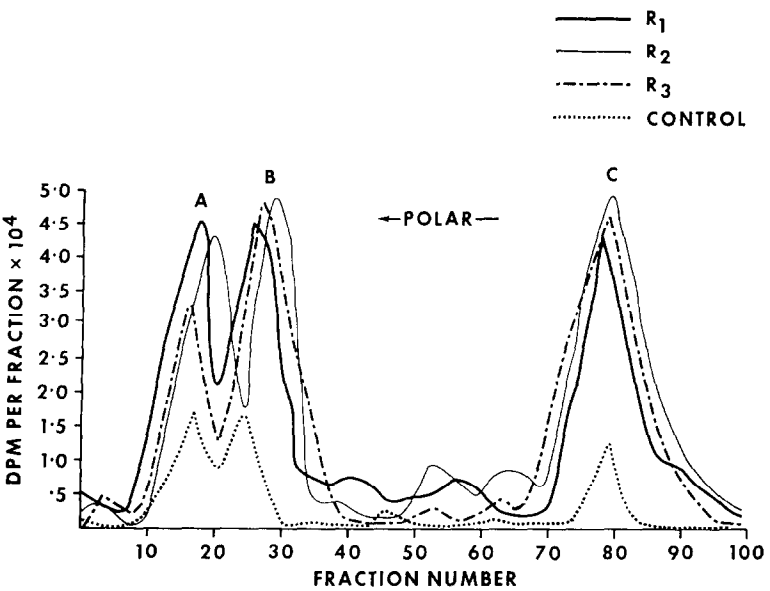

FIG. 1. Radiochromatograms obtained by countercurrent distribution of urine from the control rabbit and the rabbits receiving large doses of digitoxin-7αT.

TABLE 2. PER CENT DISTRIBUTION OF RADIOACTIVITY RECOVERED UNDER THE 3 MAJOR PEAKS FROM COUNTERCURRENT DISTRIBUTION AND THEIR CALCULATED K VALUES

Rabbit	Countercurrent distribution peaks and respective K values*					
	A (%)		B (%)		C (%)	
R_1	24·1	(0·22)	27·3	(0·34)	37·6	(3·71)
R_2	19·0	(0·24)	24·6	(0·41)	44·6	(4·21)
R_3	19·7	(0·18)	30·6	(0·38)	48·1	(4·21)
Control	31·2	(0·21)	27·4	(0·32)	20·1	(3·31)

* The K values are in parentheses.

TABLE 3. CALCULATED MINIMUM LETHAL DOSE (MLD) OF EACH COMPOUND*

Compound	MLD (mg/kg)†
Digitoxin	0·280 ± 0·014
Ouabain	0·083 ± 0·002
Peak A	0·080 ± 0·004
Peak B	0·074 ± 0·001

* Four cats per determination.
† MLD ± S.E.

Analysis of the paper chromatograms of the various peaks, described under Method, revealed identical chromatographic characteristics, in both systems, of the major polar peaks obtained from the test rabbits and from the control.

Bioassay. The calculated minimum lethal doses (MLD) are given in Table 3. No animal required more than 23 ml of infusion fluid nor longer than a 40-min infusion period to expire. It is evident that the two major isolated metabolites of digitoxin, Peak A and Peak B, were considerably more toxic than the parent compound.

DISCUSSION

We previously studied the metabolism of digitoxin in rabbits with heart failure and observed that an impairment of its conversion to polar metabolites occurred simultaneously with a reduction in the rate of urinary excretion of radioactivity.[8] In the present study, isolation of the two polar metabolites was facilitated by giving large quantities of digitoxin-7αT to rabbits that received KCl prior to and during glycoside administration. The ameliorating effect of KCl on cardiac glycoside toxicity is well known, although the mechanism is still not clear. Marcus *et al.*[10] have suggested that the potassium ion, given prior to cardiac glycosides, competes for similar myocardial receptors. Our data indicate that the metabolism of digitoxin is probably not qualitatively altered by prior and concomitant administration of KCl (Table 2).

Isolation of two of these polar metabolites in fairly large quantities in the present study provided adequate material to compare the toxicities of the metabolites to the parent compound, digitoxin. Results given in Table 3 indicate that the two metabolites isolated are approximately three to four times more toxic than digitoxin, with their toxicity being very similar to that of ouabain. This finding is significant because drugs are usually converted to more polar and less active compounds.[11–13]

It is interesting to compare the metabolism of cardiac glycosides in rabbits with that reported for other species. It is now apparent that the metabolism of digitoxin by rabbits does not reflect that seen in other species. In the dog, for example, digitoxin is hydroxylated at the 12-β position (digoxin) and is excreted in the urine.[14] This product is chloroform soluble, whereas our Peak A and Peak B are not. Rat and man have been shown to metabolize cardiac glycosides by a stepwise removal of the attached sugar molecules with the resulting aglycone undergoing epimerisation in the 3 position.[15–18] The 3-epi-derivatives of cardiac glycosides are nearly without biological activity.[19] It appears that man also excretes in the urine highly polar metabolites of digitoxin which have not yet been completely characterized, except for digoxin which was found.[3] Another species difference has been found in the toad, which is remarkedly tolerant to digitoxin; this species fails to metabolize digitoxin at all.[20]

REFERENCES

1. G. T. Okita, P. J. Talso, J. H. Curry, Jr., F. D. Smith, Jr. and E. M. K. Geiling, *J. Pharmac. exp. Ther.* **115**, 371 (1955).
2. E. Cox and S. E. Wright, *J. Pharmac. exp. Ther.* **126**, 117 (1959).
3. G. T. Okita, *Pharmacologist* **6**, 45 (1964).
4. F. I. Marcus, G. J. Kapadia and G. G. Kapadia, *J. Pharmac. exp. Ther.* **145**, 203 (1964).
5. J. E. Doherty and W. H. Perkins, *Am. Heart J.* **63**, 528, (1962).
6. J. E. Doherty, W. H. Perkins and M. C. Wilson, *Am. J. Med.* **37**, 536 (1964).

7. F. I. MARCUS, A. PETERSON, A. SALEL, J. SCULLY and G. G. KAPADIA, *J. Pharmac. exp. Ther.* **152,** 372 (1966).
8. C. L. GRIFFIN and S. H. BURSTEIN, *Biochem. Pharmac.* **16,** 447 (1967).
9. K. K. CHEN and A. L. CHEN, *J. Pharmac. exp. Ther.* **51,** 23 (1934).
10. F. I. MARCUS, G. G. KAPADIA and C. GOLDSMITH, *J. Pharmac. exp. Ther.* **165,** 136 (1969).
11. R. T. WILLIAMS, *Clin. Pharmac. Ther.* **4,** 234 (1963).
12. B. B. BRODIE, R. P. MAICKEL and W. R. JONDORF, *Fedn Proc.* **17,** 1163 (1958).
13. J. C. KRANTZ, JR. and C. J. CARR, in *The Pharmacologic Principles of Medical Research,* 7th edn, p. 17. Williams & Wilkins Co. (1969).
14. B. G. KATZUNG and F. H. MEYERS, *J. Pharmac. exp. Ther.* **154,** 575 (1966).
15. I. HERRMANN and K. REPKE, *Arch. exp. Path. Pharmak.* **248,** 351 (1964).
16. F. LAUTERBACH and K. REPKE, *Arch. exp. Path. Pharmak.* **239,** 196 (1960).
17. S. E. WRIGHT, *J. Pharm. Pharmac.* **14,** 613 (1962).
18. R. E. THOMAS and S. E. WRIGHT, *J. Pharm. Pharmac.* **17,** 459 (1965).
19. K. K. CHEN and F. G. HENDERSON, *J. Pharmac. exp. Ther.* **111,** 365 (1954).
20. I. HERRMANN, H. J. PORTIUS and K. REPKE, *Arch. exp. Path. Pharmak.* **247,** 1 (1964).