PAPERS AND ORIGINALS

Physiological Distribution of Digoxin in Human Heart

D. JOHN COLTART, HANS GEORG GÜLLNER, MARGARET BILLINGHAM, ROBERT H. GOLDMAN, EDWARD B. STINSON, SUMNER M. KALMAN, DONALD C. HARRISON

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Summary

Using the recipient's human heart removed at cardiac transplantation, the distribution of digoxin at both the cellular and subcellular level has been studied. In the presence of diffuse histological myocardial abnormalities tissue digoxin is decreased, but the subcellular distribution, presumably reflecting binding to a possible receptor site, is uniform. When the histological abnormality is focal then digoxin distribution is uniform.

These results suggest that in the presence of myocardial ischaemia plasma digoxin concentrations may not reflect total myocardial levels accurately.

Introduction

Cardiac glycosides are widely used for the treatment of patients with congestive heart failure and cardiac arrhythmias. Despite the widespread clinical use of these agents relatively little information is available about factors which regulate the response of the heart to such medication. Myocardial digoxin concentrations in digitalized patients have not been adequately characterized. During the past decade knowledge of the clinical pharmacology of the cardiac glycosides has expanded greatly since the introduction of assays capable of measuring concentrations in plasma (Lowenstein and Corrill, 1966; Lukas and Peterson, 1966; Smith *et al.*, 1969). Data defining the intra-

myocardial distribution of digoxin and its relationship to serum levels are lacking, however, primarily because of the difficulty of obtaining adequate samples of myocardial tissue for study. At Stanford University the recipient's heart removed at the time of cardiac transplantation provides adequate myocardial tissue to perform more definitive studies of the distribution of digoxin.

Though the cardiac receptor, or receptors, which mediates the pharmacological effect of the cardiac glycosides has neither been fully isolated nor fully identified evidence suggests that the sodium-potassium activated adenosine triphosphatase (ATPase) plays a role in the mechanism of action of the cardiac glycosides (Akera *et al.*, 1970; Besch *et al.*, 1970).

In this study we have identified this enzyme in a microsomal fraction of the human heart and measured the activity of the enzyme in vivo and to varying concentrations of digoxin in vitro.

Since patients undergoing transplantation in this study had end-stage cardiac disease due to either severe ischaemic heart disease or cardiomyopathy we also, therefore, correlated the effects of cardiac ultrastructure with the distribution of digoxin in the various chambers of the heart on macroscopic, subcellular, and enzymatic levels.

Patients and Methods

CLINICAL DETAILS

Patients selected as described by Clark *et al.* (1973) as appropriate recipient candidates for cardiac allograft transplantation were studied. There were six men and one woman, whose ages ranged from 42 to 54 years. The primary diagnosis was coronary artery disease in five patients and idiopathic cardiomyopathy in two. All patients needed maintenance digoxin therapy as well as large doses of diuretics before surgery. They all had been treated with digoxin for at least two years. The last dose of the patient's usual daily maintenance glycoside therapy was given five to seven hours before the removal of the recipient heart at the time of transplantation. In two patients (cases 6 and 7) their last dose of digoxin was given as tritiated digoxin (specific activity 150 $\mu\text{Ci}/\text{mg}$) intravenously four and five hours before the surgical removal of the heart. All had normal serum electrolytes, and all but one (case 2) had normal blood urea nitrogen

Divisions of Cardiology and Cardiovascular Surgery and Department of Pharmacology, Stanford University Medical Center, California 94305, U.S.A.

D. JOHN COLTART, M.D., M.R.C.P., Senior Fellow in Cardiology (Present address: Consultant Physician, St. Thomas's Hospital, London SE1 7EH)
HANS GEORG GÜLLNER, M.D., Postdoctorate Fellow in Pharmacology
MARGARET BILLINGHAM, M.B., B.S., Cardiac Pathologist
ROBERT H. GOLDMAN, M.D., Assistant Professor of Medicine
EDWARD B. STINSON, M.D., Assistant Professor of Surgery
SUMNER M. KALMAN, M.D., Professor of Pharmacology
DONALD C. HARRISON, M.D., William F. Irwin Professor of Cardiology

TABLE I—Plasma Concentrations, Total Myocardial Concentrations, and Microsomal Concentrations of Digoxin and Sodium Potassium ATPase Activity in Seven Patients

Case No.	Diagnosis	Plasma Concentration (ng/ml)	Total Myocardial Concentration (ng/g Heart)				Microsomal Concentration (ng/mg Protein)		(Na ⁺ , K ⁺) ATPase Activity (μmol Phosphate/mg Protein/hr)	
			R.A.	L.A.	R.V.	L.V.	R.V.	L.V.	R.V.	L.V.
1	Coronary artery disease	1.8	96	—	76.8	19.2	2.2	1.9	2.55	2.46
2	Coronary artery disease	2.6	54.4	59.2	67.2	75.2	2.8	2.51	3.58	2.51
3	Coronary artery disease	3.0	112	105	144	140				
4	Idiopathic congestive cardiomyopathy	1.4	65	42.8	65.8	76				
5	Coronary artery disease	1.0	45	45	80	120	1.5	1.2		
6	Coronary artery disease	2.1	72	70.9	92	80				
7	Idiopathic congestive cardiomyopathy	1.5	70.5	58	57.5	82				

R.A. = Right atrium. L.A. = Left atrium. R.V. = Right ventricle. L.V. = Left ventricle.

concentrations at the time of surgery. The patient in case 2 had a blood urea nitrogen level of 60 mg/100 ml at the time of surgery and "toxic" plasma concentrations of digoxin (2.6 ng/ml) immediately before surgery. The patient in case 3 also had a plasma digoxin concentration in the toxic range. Venous blood was taken into heparin tubes after induction of anaesthesia but before intravenous infusions had been set up or cardiopulmonary bypass initiated.

The heart was removed by the standard surgical procedure used by our group, which has been discussed elsewhere (Stinson *et al.*, 1969). The heart was quickly divided into its respective chambers, wiped dry then quick-frozen in dry ice to await pharmacological, biochemical, and histological analysis.

PHARMACOLOGICAL METHODS

Tissue specimens of 1 g (wet weight) were cleaned from epicardium and endocardium and then minced with scissors. The tissue was transferred into a Kontes Duall glass tissue grinder (No. 23) with glass pestle and homogenized in 3 ml of 0.04 M phosphate buffer, pH 6.6. The homogenate was transferred to a separatory funnel, 15 ml of methylene chloride were added, and the mixture was shaken vigorously for two minutes by hand. This extraction step was repeated once. The clear methylene chloride phases were pooled, placed in a round-bottomed flask, and evaporated to dryness in a Buchi Rotavapor evaporator. The residue was redissolved in 2 ml of trometamol (TRIS)-buffered human serum albumin. Then 1 ml of the buffered human serum albumin solution containing the tissue extract was assayed for digoxin by radioimmunoassay as described by Smith *et al.* (1969). Digoxin in blood serum was assayed by the same method.

The percentage recovery from the tissues was estimated by adding known amounts of digoxin to heart tissue homogenates and determining digoxin by radioimmunoassay. Also ³H-digoxin was added to homogenates and the digoxin content measured after extraction. Recoveries always exceeded 90%.

Radioactivity was measured in a Nuclear-Chicago liquid scintillation counter. For determination of ³H-digoxin in heart tissue the methylene chloride phase containing the tissue extract was evaporated in a scintillation phial with nitrogen gas, Bray's solution was added, and the sample then counted. Counting efficiency was 50%. Recovery was 90%.

BIOCHEMICAL METHODS

Microsomal-bound digoxin and sodium-potassium ATPase activity was determined as previously described (Goldman *et al.*, 1973). In two patients digoxin in varying concentrations was incubated with the microsomal fraction containing (Na⁺, K⁺)-ATPase and (Mg⁺⁺)-ATPase at 37°C for 30 and 45 minutes respectively. The reaction was started after a five-minute preincubation period by the addition of potassium chloride and TRIS-ATP. After 30 and 45 minutes respectively the reaction was stopped by adding the ice-cold trichloroacetic acid. The mixture was then centrifuged at 5000 *g* for three minutes at

40°C, and 2 ml aliquots were taken for inorganic phosphate analysis. Tubes containing enzyme but no digoxin served as controls. Each analysis was performed in duplicate and results averaged. Duplicate determinations varied by less than 10%.

HISTOLOGICAL METHODS

Tissue sections were taken from immediately next to the muscle used for biochemical and pharmacological assay. The tissue was fixed in 10% buffered formaldehyde embedded in paraffin. Sections were stained with haematoxylin and eosin and Masson's trichrome stain for fibrous tissue.

The histological sections were classified into 3 grades: grade 1, minimal morphological change compared to normal (donor atrium and ventricle); grade 2, moderate-to-severe fibrosis but with the preservation of architecture; grade 3, severe fibrosis—that is, calcification with disruption or obliteration of architecture.

We noted whether the abnormalities in the histological sections were focal and the degree of involvement of the section.

Results

The individual patient plasma concentration, total myocardial concentration of digoxin, microsomal concentration, and sodium-potassium ATPase activity are shown in table I. In cases 2 and 7 an in-vitro inhibition curve to 10⁻⁸ and 10⁻⁷

TABLE II—Results of In-vitro Incubation of Digoxin with ATPase at 37°C for 45 Minutes (Case 2) and 30 Minutes (Case 7)

	Molar Digoxin Concentration	% Inhibition of In-vivo ATPase Activity
Right ventricle	Case 2 10 ⁻⁸ 10 ⁻⁷ 10 ⁻⁸ 10 ⁻⁷	3.4
Left ventricle		73.6
		6.8
		55
Right ventricle	Case 7 10 ⁻⁸ 10 ⁻⁷ 10 ⁻⁸ 10 ⁻⁷	32
Left ventricle		85
		32
		88

TABLE III—Radioactive Counts, Total and Tritiated Digoxin Concentrations, and Total: Tritiated Digoxin Ratio in Two Patients

	c.p.m.	Total Myocardial Digoxin Concentration (ng/g Heart)	Tritiated Digoxin Concentration (ng/ ³ H)	Total: Tritiated Digoxin Ratio
		Case 6		
Right atrium	4,148	72	14.4	5
Left atrium	2,302	70.9	8	8.9
Right ventricle	2,381	92	8.29	11.1
Left ventricle	3,038	80	10.3	7.8
		Case 7		
Right atrium	2,302	70.5	8	8.8
Left atrium	1,671	58	5.8	10
Right ventricle	1,825	57.5	6.4	9.05
Left ventricle	2,764	82	9.6	8.5

TABLE IV—Histological Grades in Seven Patients

Case No.	Right Atrium		Left Atrium		Right Ventricle		Left Ventricle	
	Grade	Comments	Grade	Comments	Grade	Comments	Grade	Comments
1	2	Focal	1		1		3	About 100%
2	1		1	Subendocardial fibrosis	1		1	Subendocardial fibrosis
3	1		2	Subendocardial fibrosis	1		3	33%
4	2		2	Subendocardial fibrosis	1		1	
5	1		2	Subendocardial fibrosis	2		3	Focal
6	1		1		2		1	
7	1		1		2	Subendocardial fibrosis	2	

molar concentrations of digoxin was measured and the sodium-potassium ATPase activities are shown in table II. In cases 6 and 7 the last dose of digoxin was given as tritiated digoxin, either four or five hours before transplantation, and the radioactive counts in the four chambers of the heart, the total myocardial digoxin concentration, and the total myocardial:tritiated concentration are shown in table III. The histological grades of all seven patients are shown in table IV.

Discussion

Whereas a vast amount of knowledge has been developed relating to concentration and excretion of the cardiac glycosides very little is known of their distribution in the tissues and organs of the body. This is particularly true for the damaged heart where tissue fibrosis is common in patients given digitalis. Thus, we directed our studies towards the disposition of digoxin which is related to its distribution within and the elimination from the body. Since digoxin is given in clinical practice for its effect upon the heart it is particularly pertinent to study its distribution within the various chambers of the diseased human heart. Human cardiac transplantation provides an opportunity to study large amounts of fresh human heart tissue after the administration of drugs. Our results show clearly that when the morphological architecture of the heart is altered severely owing to a fibrotic process secondary to old myocardial ischaemia there are alterations in the myocardial content of digoxin.

Minimal general fibrosis and milder specific histological alterations in the structure do not alter the distribution between various chambers. When fibrotic tissue such as occurred in case 1 is present the content of digoxin is markedly reduced in the scar tissue. Analysis of the microsomal fraction which may represent the location of the active receptor site of digoxin, however, shows that the disparity in drug distribution is decreased even when fibrosis is severe as in case 1 and is relatively normal in other cases with fibrosis (cases 3 and 5). The discrepancy is clearly shown in case 1. In this patient there was a fourfold difference between the total concentration in the left ventricle and that in the right ventricle whereas the microsomal digoxin concentrations were quantitatively similar to the plasma concentrations. In case 2 the histological appearances were uniform throughout the four chambers of the heart as was the total myocardial concentration. In case 3 the plasma concentration at the time of transplant was high, and higher total myocardial concentrations were also observed. In case 4 the patient had idiopathic cardiomyopathy and the concentration in the four chambers of the heart was similar. In case 5 where the histological abnormality was focal in nature the total myocardial digoxin concentrations were uniformly distributed. In the two patients given labelled digoxin for their last dose the ratio of labelled to total myocardial concentration was relatively constant for all four chambers. Our results suggest that even five to seven hours after administration there is equilibration between the serum and the myocardium for all four chambers of the heart.

The enzymatic estimations of ATPase activity we performed were difficult to interpret owing to the lack of excellent control human tissue. The only control analysis we performed was on the histological appearances of the donor atria and ventricle. Caution, therefore, must be taken in extrapolating these results,

particularly in relationship to previous work of enzymatic analysis in a different species. Nevertheless, the results do have significance since they can be compared with the relative information from control animal experiments. It is difficult to differentiate the effects of digoxin on sodium-potassium ATPase activity for those secondary to the underlying myocardial disease process. In the two patients in whom in-vitro inhibition to various concentrations of digoxin was assessed, however, the enzyme was inhibited in a dose-responsive fashion. This suggests that there is still further activity of the enzyme in vivo. Gibson and Harris (1970) have reported low values for sodium-potassium ATPase in human heart samples from post-mortem myocardium. These workers found that the activity of the cardiac microsomal sodium-potassium ATPase did not differ in patients dying from cardiac disease and those dying from other causes. They interpreted this finding as supporting their previous experimental evidence that myocardial hypertrophy and prolonged administration of digitalis or diuretics had no effect on the activity of this enzyme. Other studies have not agreed with these findings in the failing heart, where decreased ATPase activity per gramme of muscle have been noted (Yazaki and Fujii, 1972).

Our results also confirm the previously reported experimental finding of an alteration in digoxin distribution in the experimental, acute, and chronically ischaemic left ventricle (Beller *et al.*, 1972). These workers found the pattern of digoxin uptake partly reflected in regional blood flow to infarcted, ischaemic, and non-ischaemic tissue. Furthermore, they speculated that quantitative differences of digoxin in infarcted myocardium combined with ischaemia may well contribute to the reported increase in electrophysiological arrhythmias seen in patients with coronary artery disease treated with cardiac glycosides.

Previous work on plasma and total myocardial digoxin concentrations have suggested that there is a constant relationship between these two and, hence, it might be expected that conclusions regarding a receptor site could be extrapolated from a plasma concentration (Doherty *et al.*, 1967). Our results tend to suggest that such conclusions would be wrong in the presence of severe myocardial fibrosis and necrosis as far as total tissue digoxin levels are concerned but not as far as microsomal-bound digoxin is concerned. There was a direct relationship between plasma and microsomal concentrations of digoxin. This study emphasizes the importance in understanding the active receptor site for the mechanism of action of a drug.

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Requests for reprints should be addressed to Dr. Coltart, St. Thomas's Hospital, London SE1 7EH.

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Variations in Carboxyhaemoglobin Levels in Smokers

C. M. CASTLEDEN, P. V. COLE

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Summary

Three experiments on smokers have been performed to determine variations in blood levels of carboxyhaemoglobin (COHb) throughout the day and night and whether a random measurement of COHb gives a true estimation of a smoker's mean COHb level. In the individual smoker the COHb level does not increase gradually during the day but is kept within relatively narrow limits. Moderately heavy smokers rise in the morning with a substantially raised COHb level because the half life of COHb is significantly longer during sleep than during the day. Women excrete their carbon monoxide faster than men. A random COHb estimation gives a good indication of the mean COHb level of an individual.

Introduction

The inhalation of tobacco smoke containing up to 5% carbon monoxide can substantially raise the level of carboxyhaemoglobin (COHb) in the blood (Goldsmith and Landaw, 1968; Cole *et al.*, 1972). Levels of up to 16% have been recorded in cigarette smokers (Lawther and Commings, 1970), and the COHb level of inhaling smokers is significantly higher than that of non-smokers (Castleden and Cole, 1974). The COHb level can thus identify those people at risk from any of the diseases associated with the inhalation of tobacco smoke even if carbon monoxide is not directly implicated in the pathogenesis of those diseases. For such identification, however, a doctor must know what relationship the random COHb estimation in patients at risk bears to the average COHb level throughout the day, to the time interval from the last cigarette, and to the activity of the patient.

Astrup (1972) has shown that a much greater degree of atherosclerosis can be induced in the aortas of cholesterol-fed rabbits when exposed to continuous carbon monoxide and air rather than to air alone. As man eats a substantial amount of cholesterol he may be subjecting himself to similar pathogenic stimuli as the rabbits if he maintains a constant level of COHb.

We therefore investigated the variation in the COHb of a moderately heavy smoker throughout 24 hours, the relationship between the rate of elimination of COHb and level of activity, and whether a random COHb estimation in a smoker gives a true estimation of his mean COHb level.

Methods

Four smokers, two men and two women, were studied for 12 hours on one day. Their COHb was estimated immediately before and after each cigarette, but they smoked entirely as they wished. They did not smoke from the time they went to bed until an indwelling venous cannula was inserted the next morning on arrival in the laboratory.

A further six volunteers had several blood samples taken on one day. They were allowed to smoke as they wished, and they carried on their normal daily activities. Each subject chose when he provided the blood sample so long as it was not within one hour of the previous sample or within half an hour of having smoked a cigarette.

Finally, nine subjects, four men and five women, were asked to smoke their usual number of cigarettes between waking and 11.00 hours. They then had no further cigarettes for four hours and, after 30 minutes, blood samples were taken every half an hour. Light activity involving moving around a room was allowed in this period. After four hours they were allowed to smoke as they wished. Each subject was provided with a means of collecting a further sample of blood immediately before sleep and immediately on waking the next morning.

All volunteers regularly smoked more than 10 cigarettes a day. All COHb values were estimated from venous blood samples as previously described (Castleden and Cole, 1973).

Results

The mean initial COHb level in the morning of the four subjects in the first experiment was 3.2%. Though the COHb invariably rose with every cigarette smoked (mean rise 1.3%) and always

FIG. 1—Effect of cigarette smoking on carboxyhaemoglobin (COHb) level in four subjects. Levels were estimated immediately before and after each cigarette.

St. Bartholomew's Hospital, London EC1A 7BE

C. M. CASTLEDEN, M.R.C.P., Research Registrar, Department of Clinical Pharmacology (Present address: Southampton General Hospital, Southampton SO9 4XY)

P. V. COLE, F.F.A. R.C.S., Consultant Anaesthetist