Distribution of digoxin in the human heart

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DATA defining the intramyocardial distribution of digoxin and its relationship to serum levels are lacking primarily owing to the limitation of an adequate supply of myocardial tissue for study. By using the recipient heart removed at the time of cardiac transplantation a supply of myocardial tissue is realized for more definitive studies of the distribution of digoxin, as reported in this study.

Although the cardiac receptors which mediate the pharmacological effect of the cardiac glycosides have neither been fully isolated nor fully identified, evidence suggests that the sodium potassium-activated adenosine triphosphatase, (Na^+ , K^+)-ATPase, plays a role in the mechanism of action of the cardiac glycosides (Aker, Larsen and Brody, 1970; Besch *et al.*, 1970). In this study, we have identified this enzyme in a microsomal fraction of the heart and measured the activity of the enzyme *in vivo* and to varying concentration of digoxin *in vitro*. Furthermore, patients undergoing transplantation in this study had end-stage cardiac disease due either to severe ischaemic heart disease or cardiomyopathy; we have, therefore, correlated the effects of cardiac histology with the distribution of digoxin in the various chambers of the heart, both on a subcellular and enzymatic level.

Clinical details

Patients considered as appropriate recipient candidates for cardiac allograft transplantation were selected for study as described by Clark *et al.*, 1973. Six were male and one female, ages ranging between 42 and 54 years; the primary diagnosis was coronary artery disease in five patients and idiopathic cardio-

myopathy in two. All patients required maintenance digoxin therapy as well as large doses of diuretics before surgery. They all had been treated with digoxin for a minimum of 2 years. The last dose of the patient's usual daily maintenance glycoside therapy was given 5-7 hr before removal of the recipient heart at the time of transplantation. In patients 6 and 7, their last dose of digoxin was given as tritiated digoxin (specific activity 150 $\mu\text{Ci}/\text{mg}$) intravenously 4 and 5 hr before surgical removal of the heart. All had normal serum electrolytes and all but one (patient 2) had normal blood urea nitrogen concentrations at the time of surgery. Patient 2 had a blood urea nitrogen level of 60 $\text{mg}\%$ at the time of surgery and 'toxic' plasma concentrations of digoxin before surgery. Venous blood was taken into heparin tubes after induction of anaesthesia but before intravenous infusions had been set up or cardio-pulmonary bypass initiated.

The heart was removed by the standard surgical procedure used by our group and has been discussed elsewhere (Stinson *et al.*, 1969). The heart was quickly divided into its respective chambers, dried and immediately frozen 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 mol phosphate buffer, pH 6.6. The homogenate was transferred to a separatory funnel, 15 ml of methylene chloride were added, and the mixture shaken

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vigorously for 2 min by hand. This extraction step was repeated once. The clear methylene chloride phases were pooled, placed in a round bottom flask and evaporated to dryness in a Buchi Rotavapor evaporator. The residue was redissolved in 2 ml of tris-buffered human serum albumin (BHSA). One millilitre of the BHSA solution, containing the tissue extract, was then assayed for digoxin by radioimmunoassay as described by Smith, Butler and Haber (1969). Digoxin in blood serum was assayed by the same method.

The per cent 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, model Unilux.

For determination of ^3H -digoxin in heart tissue the methylene-chloride phase containing the tissue extract was blown down in a scintillation vial with nitrogen gas, Bray's solution was added and the sample then counted. Counting efficiency was 50%. Recovery, checked by external standardization, was 90%.

Biochemical methods

Microsomal-bound digoxin and sodium-potassium ATPase activity was determined as previously described (Goldman *et al.*, 1973).

Histological methods

Tissue sections were taken immediately adjacent to the muscle used for biochemical and pharmaco-

logical assay. The tissue was fixed in 10% buffered formaldehyde embedded in paraffin. Sections were stained with both haematoxylin and eosin and Masson's trichrome stain for fibrous tissue. The histological sections were classified into three grades: Grade 1—minimal morphological change compared to normal (donor atrium and ventricle); Grade 2—moderate-severe fibrosis but with the preservation of architecture; Grade 3—severe fibrosis—calcification with disruption or obliteration of architecture. From the sections, note was made as to whether the abnormalities were focal or the degree of involvement of the section.

Results (Tables 2–8 give individual patients' results)

The individual patient plasma concentration and total myocardial concentration of digoxin are enumerated. In some patients, the microsomal concentration in both the right and left ventricle was measured and/or sodium-potassium ATPase activity. In patients 2 and 7 an *in vitro* inhibition curve to 10^{-8} and 10^{-7} molar digoxin was performed. In patients 6 and 7, the last dose of digoxin was given as tritiated digoxin, either 4 or 5 hr before transplantation and the radioactive counts in the four chambers of the heart, the total myocardial digoxin concentration and the total myocardial-tritiated concentration ratio are shown. The histology index for all seven patients is shown in Table 1.

Discussion

Whereas a vast amount of knowledge has accrued relating to the excretion of the cardiac glycosides, little is known of the distribution of the drug within the body. The disposition of any drug is related to

TABLE 1. Histology grade

Patient	Right atrium		Left atrium		Right ventricle		Left ventricle		Septum	
	Grade	Comments	Grade	Comments	Grade	Comments	Grade	Comments	Grade	Comments
1 J.H.	2	Focal	1	—	1	—	3	Approx. 50%	3	50%
2 W.S.	1	—	1	Sub-endocardial fibrosis	1	—	1	Sub-endocardial fibrosis	2	33%
3 G.M.	1	—	1	Sub-endocardial fibrosis	1	—	3	33%	2	Sub-endocardial fibrosis
4 R.N.	2	—	2	Sub-endocardial fibrosis	1	—	1	—	2	33%
5 G.O.	1	—	2	Sub-endocardial fibrosis	2	—	3	Focal	2	—
6 R.E.	1	—	1	—	2	—	1	—	3	90%
7 K.C.	1	—	1	—	2	Sub-endocardial fibrosis	2	—	2	—

TABLE 2. Patient 1. J.H. 52-year-old male.
Coronary artery disease, antero-inferior infarction

Plasma concentration		
	1.8 ng/ml	
Myocardial concentration		
RA	96.0 ng/g	Heart
RV	76.8 ng/g	Heart
LV	19.2 ng/g	Heart
Microsomal concentration		
RV	2.2 ng/mg	Protein
LV	1.9 ng/mg	Protein
Na ⁺ K ⁺ ATP activity		
RV	2.55 μ mol Pi/mg protein/hr	
LV	2.46 μ mol Pi/mg protein/hr	

TABLE 3. Patient 2. W.S. 53-year-old male.
Coronary artery disease, inferior infarction

Plasma concentration date		
2/23	4.2 ng/ml	
2/24	3.1 ng/ml	
2/25	2.6 ng/ml	Day of transplant
Myocardial concentration		
RA	54.4 ng/g	Heart
LA	59.2 ng/g	Heart
RV	67.2 ng/g	Heart
LV	75.2 ng/g	Heart
Microsomal concentration		
RV	2.8 ng/mg	Protein
LV	2.51 ng/mg	Protein
Na ⁺ K ⁺ ATPase activity		
RV	3.58 μ mol Pi/mg protein/hr	
LV	2.51 μ mol Pi/mg protein/hr	
<i>In vitro</i> inhibition 45 min at 37°C		
	Digoxin concentration	Inhibition of <i>in vivo</i> activity %
RV	10 ⁸ -	3.4%
	10 ⁷ -	73.6%
LV	10 ⁸ -	6.8%
	10 ⁷ -	55.0%

TABLE 4. Patient 3. G.M. 48-year-old male.
Coronary artery disease antero-septal infarct, apical aneurysm

Plasma concentration 3.0 ng/ml		
Myocardial concentration	RA	112 ng/g
	LA	105 ng/g
	RV	144 ng/g
	LV	140 ng/g

TABLE 5. Patient 4. R.N. 42-year-old male.
Idiopathic cardiomyopathy

Plasma concentration 1.4 ng/ml		
Myocardial concentration	RA	65.0 ng/g heart
	RV	42.8 ng/g heart
	IVS	65.8 ng/g heart
	LV	76.0 ng/g heart

TABLE 6. Patient 5. G.O. 47-year-old male.
Coronary artery disease, anterior and interior infarction

Plasma concentration 1.0 ng/ml	
Myocardial concentration	
RA	45 ng/g
LA	45 ng/g
RV	80 ng/g
LV	120 ng/g
Microsomal concentration	
RV	1.5 ng/mg protein
LV	1.2 ng/mg protein

TABLE 7. Patient 6. R.E. 54-year-old male.
Coronary artery disease, antero-septal infarction.
0.25 mg ³H digoxin i.v. 5 hr pre-TS

Plasma concentration 2.1 ng/ml			
	Counts/min	ng/g heart	ng ³ H
RA	4148	72.0	14.4
LA	2302	70.9	8.0
RV	2381	92.0	8.29
LV	3038	80.0	10.3
			Ratio
			5.0
			8.9
			11.09
			7.8

TABLE 8. Patient 7. K.C. 49-year-old female.
Cardiomyopathy. 0.1 mg ³H digoxin i.v. 4 hr pre-TS

Plasma concentration 1.5 ng/ml			
	Counts/min	ng/g heart	ng ³ H
RA	2302	70.5	8.0
LA	1671	58.0	5.8
RV	1825	57.5	6.4
LV	2764	82.0	9.6
			Ratio
			8.8
			10.0
			9.05
			8.5
<i>In vitro</i> inhibition 30 min at 37°C			
	digoxin concentration	Na ⁺ K ⁺ ATPase active	Inhibition of <i>in vivo</i> activity %
RV	10 ⁻⁸ mol digoxin	5.5	32%
	10 ⁻⁷ mol digoxin	1.25	85%
LV	10 ⁻⁸ mol digoxin	4.35	32%
	10 ⁻⁷ mol digoxin	0.74	88%

its distribution within and the elimination from the body. As digoxin is mainly given in clinical practice for its effect upon the heart, it is particularly pertinent to study the distribution of this drug within the diseased human heart. Human cardiac transplantation offers an opportunity to study large amounts of fresh human heart tissue. The results of this study clearly show that when the morphological architecture of the heart is altered owing to a fibrotic process secondary to old myocardial ischaemia, then the total myocardial content of digoxin is decreased. However, when on examining a purer fraction representing the possible active receptor site location of digoxin, then the disparity in drug distribution is decreased.

In patient 1 there was a fourfold difference between the total concentration in the left ventricle and right ventricle, whereas the microsomal digoxin concentrations were qualitatively similar to the plasma concentration; in patient 2 the histology was uniform

throughout the four chambers of the heart as was the total myocardial concentration, possibly representing the relative blood supply to these four chambers; in patient 3 the plasma concentration at the time of transplant was high, higher total myocardial concentrations were also observed; in patient 4 (with idiopathic cardiomyopathy) the concentrations in the four chambers of the heart again possibly represent the blood distribution as also seen in patient 5 where the histology was focal in nature and hence did not cause any alteration in digoxin distribution. In the two patients given their last dose as labelled digoxin, the ratio of labelled to total myocardial concentration is relatively constant for all four chambers, suggesting a rapid equilibration of serum and myocardial digoxin.

The enzymatic estimations performed in this study are difficult to interpret owing to the lack of control human tissue. The only control analysis performed in this study was histology of the donor atria and ventricle. Caution has therefore to 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 upon sodium-potassium ATPase activity from those secondary to the underlying myocardial disease process. However, in the two patients in whom *in vitro* inhibition to various concentrations of digoxin was performed, the enzyme could be inhibited in a dose responsive fashion. This would tend to indicate that there is still further activity of the *in vivo* situation. Gibson and Harris (1970) reported low values for sodium-potassium ATPase from human heart samples taken from post-mortem myocardium. These workers found that the activity of the cardiac microsomal sodium-potassium ATPase did not differ, in subjects dying from cardiac disease, from those dying from other causes. They interpreted this finding to support their previous experimental evidence that myocardial hypertrophy and prolonged administration of digitalis or diuretics had no effect on the activity of this enzyme.

This present study confirms the previously reported experimental finding of an alteration in digoxin distribution in the chronically ischaemic and diseased left ventricle (Beller, Smith and Hood, 1972). It

highlights the importance of an understanding of an active receptor site for the action mechanism of a drug. Previous work in relationship to plasma-total myocardial digoxin concentrations (Doherty, Perkins and Flanigan, 1967) has suggested that there is a constant relationship between these two, and hence conclusions regarding a receptor site could be extrapolated from a plasma concentration. The results of this study would tend to indicate a fallacy of such a viewpoint.

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