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The Renal Clearance of Digoxin is Dependent upon the Serum Digoxin Concentration[1]

THOMAS P. GIBSON and ANTONIO P. QUINTANILLA

Section of Nephrology/Hypertension and Department of Medicine, Northwestern University Medical School. Northwestern Memorial Hospital and Veterans Administration Lakeside Medical Center, Chicago, Illinois

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ABSTRACT

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The effect of alterations of serum digoxin concentrations on the renal clearance of digoxin (C_{DIG}) was studied in seven dogs. Digoxin, 0.016 μg/kg/min (low dose) in normal saline was infused at the rate of 1 ml/min. After 60 min of equilibration, five 15-min urine collections were made. The digoxin infusion was then increased to 0.112 μg/kg/min (high dose) and five additional collections were made after equilibration. Digoxin was measured by ^{125}I radioimmunoassay whose specificity was confirmed by high-pressure liquid chromatography. With low dose digoxin, the serum digoxin concentration was 0.5 ± 0.2 (S.D.) ng/ml, C_{DIG} 58 ± 26 ml/min, inulin clearance (C_{IN}) 48 ± 8 ml/min and C_{DIG}/C_{IN} 1.2 ± 0.5. With high dose infusion, the serum digoxin concentration rose to 5.2 ± 1.4 ng/ml, C_{DIG} decreased 43% to 33 ± 13 ml/min and C_{DIG}/C_{IN} decreased 48% to 0.6 ± 0.2 ($P < .05$ for both compared to control, while C_{IN} remained constant. We conclude that C_{DIG} is not as dependent upon glomerular filtration as previously thought. Increases in serum digoxin concentrations can significantly reduce C_{DIG} without altering glomerular filtration rate.

The renal handling of digoxin in man is complex. Digoxin renal clearance in man has been closely correlated with creatinine clearance (Bloom and Nelp, 1966; Doherty and Perkins, 1962; Jelliffe, 1967) and such a correlation serves as the basis of a commonly used digoxin dosing nomogram (Jelliffee and Booker, 1974). These observations suggested that digoxin was primarily excreted by filtration. However, Steiness (1974) demonstrated in man that net tubular secretion of digoxin can occur which can be diminished by spironolactone. Quinidine reduces the renal clearance of digoxin out of proportion to any change in creatinine clearance (Hooymans and Merkus, 1978; Doering, 1979; Dahlquist *et al.*, 1980), probably by reducing net digoxin tubular secretion. Halkin and associates (1975) showed that digoxin renal clearance was significantly related to urine flow rate, independent of creatinine clearance, and suggested that digoxin undergoes some degree of tubular reabsorption. These studies indicate that factors other than glomerular filtration may be important determinants of the renal clearance of digoxin.

In dogs, the renal clearance of digoxin may also be related to creatinine clearance (Gierke *et al.*, 1978; Risler *et al.*, 1980). Risler and associates (1980) reduced renal arterial pressure in dogs which reduced not only the glomerular filtration rate but also the renal clearance of digoxin. However, the digoxin-to-inulin clearance ratio remained constant and they concluded that the renal excretion of digoxin closely approximates the filtered load of digoxin.

In the present study, the filtered load of digoxin was altered by increasing the serum digoxin concentration (S_{DIG}) 10-fold. As the S_{DIG} increased the ratio of digoxin clearance (C_{DIG}) to inulin clearance (C_{IN}) decreased 48% while C_{IN} remained constant, indicating that the renal clearance of digoxin in the dog may not be as dependent upon glomerular filtration as previously thought.

Methods

Seven mongrel dogs [13.9 ± 2.3 kg (S.D.)] were anesthetized with i.v. pentobarbital, 30 mg/kg, and intubated with a cuffed endotracheal tube. Anesthesia was maintained with intermittent small doses of less than 30 mg of pentobarbital. The jugular veins were catheterized bilaterally for infusion of digoxin and saline. A femoral artery and vein were cannulated for blood sampling and administration of inulin and *p*-aminohippurate (PAH). Through a suprapubic incision, the ureters were isolated and catheterized.

Inulin, 50 mg/kg, and PAH, 10 mg/kg, were given as a bolus followed by a sustaining infusion (0.4 ml/min) to maintain serum inulin and PAH concentrations between 0.19 and 0.26 mg/ml and between 0.010 and 0.015 mg/ml, respectively. Normal saline, 0.7 ml/kg/min, was infused into one jugular vein.

Digoxin (Lanoxin), 0.016 μg/kg/min (low dose) in normal saline, was infused at a rate of 1 ml/min into the other jugular vein. After 60 min

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of equilibration, five urine collections with midcollection blood samplings were made. All urine collections for all phases of this study were 15 min in duration. At the conclusion of the fifth period, the digoxin infusion was changed to 0.112 μg/kg/min (high dose) infused in normal saline at a rate of 1 ml/min. After an hour of equilibration, five additional urine collections were made. The rate of digoxin infusion kept constant for the duration of the study.

All digoxin analyses were by radioimmunoassay (RIA) performed in duplicate as previously described (Gibson and Nelson, 1980). Urine samples were diluted with buffer from the RIA kit [^{125}I RIA Ab-TRAC (T.M.) Digoxin Solid Phase Radioimmunoassay Kit, Becton Dickinson Immunodiagnostics (Orangeburg, NY) which had been diluted to 400 ml with distilled water containing 2% propylene glycol and 5% blank canine plasma. The coefficient of variation, $N = 10$, in urine and serum was less than 7% over the range of the standard curve (0.2 to 5.0 ng/ml).

The quantitative assessment of renal transport requires chemical measurements that are accurate and specific. Digoxin RIA antibodies are nonspecific and react with the digoxin metabolites digoxin-bis-digitoxoside and digoxigenin-mono-digitoxoside (Stoll *et al.*, 1972). The specificity of the RIA was confirmed by separating digoxin from its metabolites by high-pressure liquid chromatography (HPLC) (Nelson *et al.*, 1979). The procedure has been modified from the original description (Nelson *et al.*, 1979) by using a 4.6 × 150 mm Altex Ultrasphere ODS reverse phase column in place of a Waters Associates C-18 μBondapak column. There was less than 6% difference between digoxin concentrations estimated by RIA alone and that determined after removal of metabolites by HPLC. These results are consistent with previous findings (Gibson and Nelson, 1980) and indicate that under the conditions of these experiments conventional digoxin RIA can be used to accurately assess digoxin renal transport.

Digoxin protein binding. Digoxin protein binding was determined by equilibrium dialysis of serum samples taken from each dog during each digoxin infusion. Tritiated digoxin (New England Nuclear, Boston, MA), 48 × 10^3 dpm or approximately 0.8 ng, was added to 0.8 ml of 0.125 M isotonic sodium phosphate buffer. Before use, the tritiated digoxin was chromatographed by thin-layer chromatography with chloroform-methanol-ammonium hydroxide (9:1:1) and by HPLC. Compared to pure digoxin (Boehringer Mannheim Biochemicals, Indianapolis, IN), 97% or more of the label represented digoxin. All serum digoxin concentrations are reported as the total (bound + unbound) digoxin concentration.

Canine serum (0.8 ml) was dialyzed against an equal volume of buffer at 37°C for 20 hr in Plexiglass cells separated by a cellophane membrane (Spectrapor, Scientific Products, McGaw Park, IL). Preliminary experiments indicated that dialysis equilibrium was reached in 16 hr when the drug was added to either the buffer or serum side of the system. Tritium was counted using a Beckman model LS 8100 Scintillation Counter.

Inulin was measured by by the method of Schreiner (1960) and PAH by the method of Smith *et al.* (1945).

C_{DIG} was calculated in the standard manner using $f \cdot S_{DIG}$ where f is the unbound fraction of the total serum digoxin concentration.

Statistical significance was determined by using a two-tailed paired Student's *t* test. The minimal level of significance was taken as a P value of <.05. Results in text and tables are given as mean ± 1 S.D.

Results

With low dose digoxin infusion, the S_{DIG}s were either steady or very slowly rising during the five collection periods and the mean value was 0.52 ± 0.17 ng/ml (table 1). Increasing the concentration infused by a factor of 7 resulted in a 10-fold increase in the mean S_{DIG}s to 5.24 ± 1.45 ng/ml.

During the low dose infusion, the mean C_{DIG} averaged 57.9 ± 26.1 ml/min and decreased to 33.1 ± 12.7 ml/min ($P < .05$) with the high dose, whereas C_{IN} remained constant (tables 1 and 2). In two dogs, 346 and 356, tables 1 and 2, C_{DIG} increased from 29.8 and 33.7 ml/min to 41.1 and 52.6 ml/min, respectively. In both dogs, C_{IN} increased in the high dose period 47 and 13% respectively, compared to the low dose period. In dog 335, C_{IN} increased 25% but C_{DIG} decreased 55%.

During the high dose infusion, the fractional clearance (C_{DIG}/C_{IN}) decreased from 1.20 to 0.62, $P < .02$ (table 1). A decrease in C_{DIG}/C_{IN} was noted in all dogs except dog 356. The mean C_{DIG}/C_{IN} was greater than 1 during the low dose infusion. During high dose infusion, C_{DIG}/C_{IN} was less than 1 in all dogs.

C_{PAH} fell from 145 ml/min to 125 ml/min ($P < .05$) with high dose digoxin (table 2). V was essentially constant throughout the study (table 2). Serum digoxin protein binding decreased slightly from 22.6% during low dose infusion to 20.7% with high infusion (table 3).

Discussion

This study demonstrates that the renal clearance of digoxin in the dog is inversely related to serum digoxin concentration at least under the conditions of this study. This observation indicates that the renal clearance of digoxin may not be as dependent upon glomerular filtration as previously thought (Bloom and Nelp, 1966; Doherty and Perkins, 1962; Jelliffe, 1967; Gierke *et al.*, 1978; Risler *et al.*, 1980). A 10-fold increase in mean S_{DIG} was accompanied by a 43% decrease in mean C_{DIG} while C_{IN} remained constant. If C_{DIG} were solely a function of C_{IN} the renal clearance of digoxin and C_{DIG}/C_{IN} would be predicted to remain constant over a wide range of S_{DIG}s. This is clearly not the case. The extent of the reduction in C_{DIG} and C_{DIG}/C_{IN} associated with high S_{DIG}s varied markedly (table 1; fig. 1). In dog 352, C_{DIG} fell 72% from 107 to 30 ml/min while C_{IN} remained virtually constant. On the other hand, in dogs 346 and 356, C_{DIG} increased 13 and 28%, respectively, as the S_{DIG} increased. C_{IN} increased 47% in dog 346 and 13% in dog 356, but C_{DIG}/C_{IN} decreased 26% in dog 346 and increased 15% in dog 356 during the same period of time. Thus, changes in glomerular filtration rate may be accompanied by similar directional changes in C_{DIG}. These results are consistent with the previous notion that changes in glomerular filtration rate are accompanied by similar changes in C_{DIG} (Gierke *et al.*, 1978). Recently, Risler and associates (1980) showed that the fall in C_{DIG} was commensurate with a reduction in C_{IN} (C_{DIG}/C_{IN} remained constant) produced by partial clamping of the renal artery. The S_{DIG}s in the study of Risler and associates (1980) were 3 ng/ml or greater. As can be seen in figure 1, as the S_{DIG} exceeds 3 ng/ml, the clearance of digoxin is less than the clearance of inulin, indicating net digoxin reabsorption. It may be that at these S_{DIG}s, C_{DIG} will vary directly and predictably with glomerular filtration rate. In goats, the fractional clearance of digoxin also varies inversely with the S_{DIG} (Rasmussen *et al.*, 1975), but the effect of changes of concentration in individual animals was not examined.

Digoxin renal clearance is not solely dependent upon glomerular filtration. This conclusion is indicated by the fact that in at least three of our dogs there was apparent net digoxin secretion, $C_{DIG}/C_{IN} > 1.0$, during the low dose infusion (fig. 1; table 1), whereas in the remainder of the dogs, net digoxin reabsorption, $C_{DIG}/C_{IN} < 1.0$, was observed. The magnitude of digoxin secretion can be quite large as shown by a fractional C_{DIG} of 2.08 in dog 352 during the low dose infusion. In goats, the fractional clearance of digoxin approaches 2.0 at digoxin

TABLE 1

S_{DIG}, C_{DIG} and C_{DIG}/C_{in} during low and high dose digoxin infusion

Dog No.	Weight	Sex	S_{DIG}		C_{DIG}		C_{DIG}/C_{in}	
			Low	High	Low	High	Low	High
	kg		ng/ml		ml/min			
333	16.3	M	0.63 ± 0.07	5.93 ± 0.35	66.5 ± 6.8	45.8 ± 6.4	1.47 ± 0.23	0.99 ± 0.06
340	12.7	F	0.37 ± 0.04	6.38 ± 0.70	69.7 ± 9.7	30.7 ± 3.6	1.39 ± 0.13	0.67 ± 0.06
335	16.8	M	0.40 ± 0.03	5.26 ± 0.25	53.4 ± 9.1	24.2 ± 5.1	1.09 ± 0.18	0.38 ± 0.09
346	14.5	F	0.50 ± 0.04	5.04 ± 0.34	29.8 ± 6.3	33.7 ± 2.9	0.83 ± 0.05	0.61 ± 0.07
356	15.0	F	0.59 ± 0.11	3.29 ± 0.86	41.1 ± 10.0	52.6 ± 5.2	0.61 ± 0.06	0.70 ± 0.10
336	11.8	M	0.81 ± 0.07	7.26 ± 0.57	37.9 ± 5.1	14.9 ± 4.6	0.94 ± 0.10	0.31 ± 0.05
352	10.5	M	0.31 ± 0.03	3.51 ± 0.03	106.9 ± 1.7	29.7 ± 3.9	2.08 ± 0.18	0.66 ± 0.08
Mean ± 1 S.D.			0.52 ± 0.17	5.24 ± 1.45**	57.9 ± 26.1	33.1 ± 12.7*	1.20 ± 0.49	0.62 ± 0.22**

* $P < .05$; ** $P < .02$.

TABLE 2

C_{IN}, C_{PAH} and V during low and high dose digoxin infusion

Dog No.	C_{IN}		C_{PAH}		V	
	Low	High	Low	High	Low	High
	ml/min		ml/min		ml/min	
333	45.4 ± 2.8	46.8 ± 2.5	177.9 ± 20.0	131.9 ± 13.7	0.24 ± 0.03	0.34 ± 0.06
340	49.9 ± 4.0	45.5 ± 1.6	163.5 ± 12.3	118.8 ± 7.1	1.13 ± 0.25	0.92 ± 0.19
335	49.8 ± 8.0	62.4 ± 6.9	141.7 ± 16.2	141.1 ± 6.8	0.29 ± 0.09	0.34 ± 0.03
346	37.4 ± 6.0	55.0 ± 1.9	110.2 ± 15.3	118.7 ± 6.7	0.31 ± 0.09	0.68 ± 0.05
356	63.9 ± 6.0	72.4 ± 12.0	162.3 ± 23.0	169.6 ± 35.5	0.13 ± 0.08	0.48 ± 0.08
336	40.8 ± 2.1	39.8 ± 1.8	131.8 ± 9.1	99.3 ± 2.2	1.58 ± 0.30	1.58 ± 0.30
352	49.5 ± 5.8	44.8 ± 5.1	129.3 ± 21.7	100.2 ± 12.5	1.27 ± 0.71	1.68 ± 0.28
Mean ± S.D.	48.1 ± 8.5	52.4 ± 11.6	145.2 ± 23.7	125.6 ± 24.7	0.73 ± 0.57	0.86 ± 0.56

TABLE 3

Serum protein binding of digoxin during low and high dose digoxin infusion

Dog No.	% Bound	
	Low dose	High dose
333	27.1	27.4
340	24.2	22.6
335	20.7	19.0
346	24.5	19.7
356	20.0	17.9
336	21.9	20.9
352	20.0	17.5
Mean ± 1 S.D.	22.6 ± 2.7	20.7 ± 3.4

concentrations of less than 1 ng/ml and falls below 1 as digoxin concentrations exceed 2.5 ng/ml (Rasmussen *et al.*, 1975). Net digoxin secretion of a similar magnitude is also evident in dogs from the data of Gierke and associates (1978) but the associated digoxin concentrations are not given. In man, the data of Steiness (1974) and Halkin and associates (1975) also show examples of net digoxin secretion but in neither report are sequential clearances with varying digoxin concentrations given. The factors affecting net digoxin secretion remain to be determined but one of them is the S_{DIG}. In six of seven dogs an increase in S_{DIG} to greater than 3 ng/ml was associated with a decrease in the fractional clearance of digoxin (table 1). In all dogs initially exhibiting net secretion, the increase in serum digoxin concentration reduced the fractional clearance to less

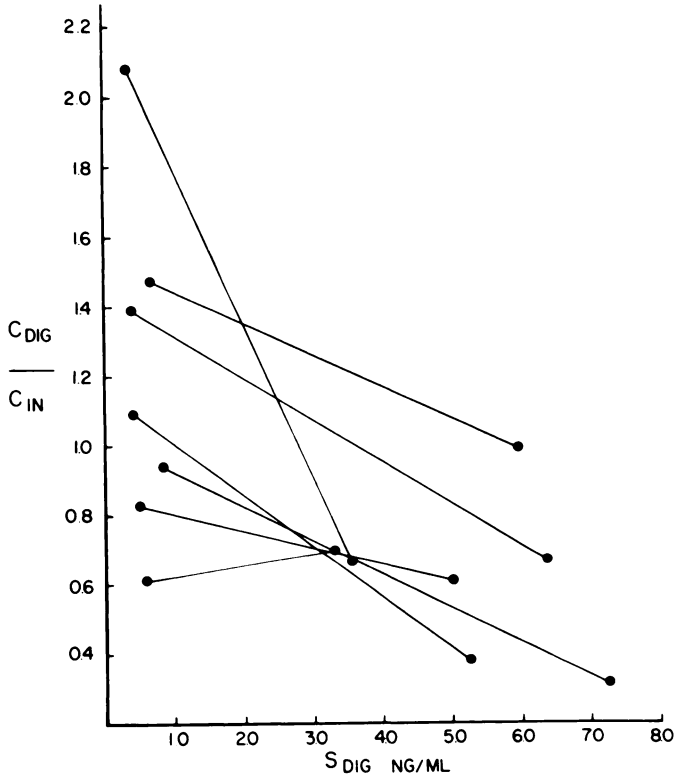

Fig. 1. The effect of alterations in S_{DIG} on the fractional clearance of digoxin (C_{DIG}/C_{IN}). Lines join points from the same dog.

than 1. Thus, it would appear that digoxin undergoes bidirectional transport with net secretion at low serum concentrations and net reabsorption at high concentrations.

The mechanism of the decrease in fractional digoxin clearance at high S_{DIG}s cannot be determined from this study. In most cases in which a substance undergoes tubular secretion, the rate of secretion is proportional to the serum concentration at low levels and renal clearance (by filtration and secretion) will not change. However, when the serum concentration is sufficiently high to saturate the tubular transport system tubular maximum (Tm), an additional increase in the substance's concentration does not result in further excretion and clearance falls. We have no evidence to support or refute this possibility for digoxin since the secretory Tm is unknown. Alternatively, it could be postulated that at high S_{DIG}s digoxin inhibits a digoxin secretory mechanism. If so, it may be further postulated that the secretory mechanism may involve Na^+-K^+-adenosine triphosphatase (ATPase) which digoxin is known to inhibit (Martinez-Maldonado *et al.*, 1972). Why only three dogs exhibited apparent net digoxin secretion initially during the low dose infusion is unknown. There is in this study no correlation between S_{DIG}, weight, sex and C_{DIG}/C_{IN}.

C_{PAH} decreased slightly in four dogs during the high infusion as compared to the low infusion. The filtration fraction rose from 0.333 at the low dose to 0.417 at the high dose. This may result from an inhibition of PAH transport by digitalis as observed by Burg and Orloff (1962) in kidney slices. However, an actual reduction in renal blood flow has not been ruled out by this study.

The protein binding of digoxin as determined by equilibrium dialysis was 22.6% at low S_{DIG}s and was slightly, but not significantly, less at the higher concentrations. These values are slightly lower than the 27% protein binding in dogs reported by Baggot and Davis (1973).

In conclusion, this study has demonstrated the renal clearance of digoxin is, in part, dependent upon the S_{DIG}.

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Send reprint requests to: Thomas P. Gibson, M.D., Renal Section, Veterans Administration Lakeside Medical Center, 333 E. Huron St., Chicago, IL 60611.