Br. J. clin. Pharmac. (1977), **4**, 223-227

MONITORING DIGOXIN THERAPY: II. DETERMINANTS OF THE APPARENT VOLUME OF DISTRIBUTION

J.K. ARONSON & D.G. GRAHAME-SMITH

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

1 In eighteen subjects receiving digoxin therapy the apparent volume of distribution (V_d) of the drug has been calculated.

2 For each subject the sensitivity of the ^{86}Rb transport mechanism of his pre-treatment erythrocytes to *in vitro* inhibition by digoxin has been measured (IC_{50}).

3 The V_d of digoxin correlates both with the age of the patients and with the IC_{50} of their pre-treatment erythrocytes.

4 The implications of these findings are discussed in terms of the possible determinants of the V_d of digoxin.

5 Digoxin requirements may be predictable before therapy commences if IC_{50} measurements are performed.

Introduction

In the course of investigating the utility of measuring a pharmacodynamic effect of digoxin on patients' own erythrocytes (the degree of diminishment of the ability of those cells to accumulate ^{86}rubidium) (Aronson, Grahame-Smith, Hallis, Hibble & Wigley, 1977) it was discovered that the calculated apparent volume of distribution of digoxin (V_d) was correlated with both the age of the patient and the *in vitro* sensitivity of the ^{86}rubidium (^{86}Rb) transport mechanism to inhibition by digoxin measured before treatment was commenced. In this paper those relationships are described and their relevance to digoxin therapy discussed.

Methods

Patients

The subjects studied were twelve of those patients whose clinical details are listed in Table 1 of Aronson *et al.*, 1977, p. 213 (patients numbers 1-4, 6, 9-15), three other patients being treated for cardiac failure in sinus rhythm and three normal volunteers who took digoxin.

Measurement of IC_{50} and radioimmunoassay

Patients' venous blood was taken into lithium heparin tubes before treatment commenced and the erythrocytes were separated and washed as described (Aronson *et al.*, 1977). Packed red cells (1 ml) were incubated for 120 min at 37°C with varying concentrations of digoxin (usually 0, 10, 20 and 40 ng/ml but in some cases also 1, 5, 100 and 1000 ng/ml) diluted in 1.5 ml potassium-free Ringer (Aronson *et al.*, 1977). These concentrations are in ng/ml of red cells plus Ringer. ^{86}Rb (0.5 ml) was then added and 60 min ^{86}Rb uptakes measured as previously described (Aronson *et al.*, 1977). The concentration of digoxin required to inhibit by 50% the uptake which occurred in the absence of digoxin has been termed the IC_{50} (Figure 1).

Plasma digoxin concentrations were measured by radioimmunoassay using 12-α-[^{3}H]-digoxin (Smith, Butler & Haber, 1969).

Pharmacokinetic and statistical calculations

The apparent volume of distribution of digoxin was calculated using the following formula:

$V_d = F.D./\beta.\tau.C_{SS}$ ml (Wagner, Northam, Alway & Carpenter, 1965)

where F = fraction of dose absorbed

D = daily maintenance dose (ng)

β = disposition rate constant = $\ln 2/T_{\frac{1}{2}\beta}$ (h^{-1})

τ = 24 (h)

C_{SS} = steady state plasma digoxin concentration (ng/ml).

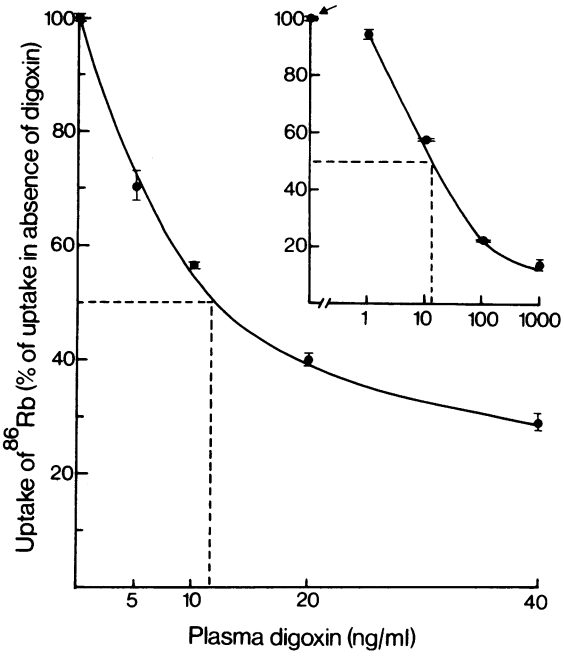

Figure 1 Dose-response curves of the *in vitro* inhibition by digoxin of the ^{86}Rb uptake of the erythrocytes of Volunteer 3. The main graph shows the response in the concentration range up to 40 ng/ml and the inset up to 1000 ng/ml (the latter on a semi-log plot).

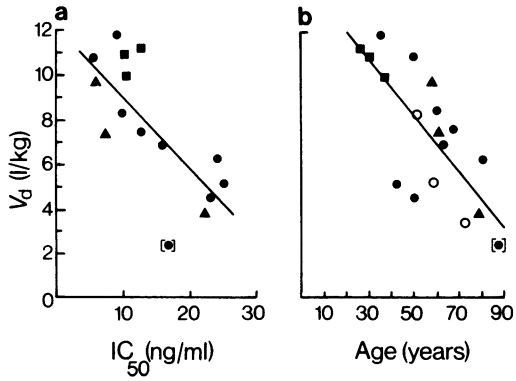

Figure 2 Relationships between apparent volume of distribution (V_d) of digoxin and (a) IC_{50} ($r = -0.7481$, $P = 0.0014$) (b) age ($r = -0.7520$, $P < 0.001$) (see text for discussion, including the relevance of the bracketed point). • and ○ numbered patients, ▲ patients A, B and C (Table 1), ■ volunteers.

C_{SS} has been taken as the average plasma concentration of at least three individual daily concentrations measured at least 6 h after the previous dose after at least one week of treatment.

D and τ are known for each patient.

F after oral dosage has been assumed to be the same for each patient and equal to 0.67 (Doherty & Kane, 1975). All digoxin tablets were Lanoxin (Burroughs Wellcome), of high bioavailability (Johnson, Bye & Lader, 1974).

$T_{\frac{1}{2}\beta}$ (the half-time of disposition) has been calculated from the ratio of daily maintenance dose to the loading dose required to produce a therapeutic effect. Thus, if the maintenance dose at steady state were 0.25 mg and the loading dose had been 0.75 mg, the ratio (1 : 3) would indicate a daily loss of one-third of the total body content at steady state and a $T_{\frac{1}{2}\beta}$ of about 41 h (and β of 0.0169). This calculation assumes a one-compartment model for the disposition of digoxin. In the case of patient A the first dose was given intravenously and serial plasma concentration measurement thereafter allowed calculation of the true V_d by the area method (Table I).

Statistical calculations have been carried out as described in Snedecor & Cochran (1967).

Results

IC_{50}

Figure 1 illustrates for volunteer 3 the dose-response curve of inhibition of ^{86}Rb uptake by digoxin in detail in the dose range up to 40 ng/ml and the method of calculating the IC_{50}. The inset shows the curve in the complete dose range up to 1000 ng/ml.

Relationships between apparent volume of distribution and (a) in vitro *pre-treatment ^{86}Rb uptake inhibition and (b) age*

Table I lists the pharmacokinetic data of the eighteen subjects, their ages and weights and the degree to which their pre-treatment red cell ^{86}Rb uptake could be inhibited by digoxin *in vitro* (expressed as IC_{50}, see **methods**).

Figure 2 illustrates the relationships between calculated apparent volume of distribution and (a) IC_{50}, (b) age. The correlations are:

(a) $r = -0.7481$ ($P = 0.0014$) and (b) $r = -0.7520$ ($P < 0.001$).

The correlation between age and IC_{50} is $r = 0.3450$ (not significantly different from zero).

When multivariate analysis of the three variables is carried out the regression equation found is:

$$V_d = 15.9 - 0.087 \text{ age} - 0.24\ IC_{50}$$

(% variation of V_d due to age and IC_{50} = 82.44%, $P < 0.001$; the coefficients of age and IC_{50} differ significantly from zero, ($P < 0.005$). The relevant partial correlation coefficients are $r = -0.7898$ ($P < 0.001$) for IC_{50} and $r = -0.7920$ ($P < 0.001$) for age.)

The bracketed point in Figure 2a differs significantly ($P = 0.01$) from the regression line computed for the other values and when it is excluded from the analysis the regression coefficient becomes $r = -0.8069$ ($P < 0.001$). This patient had a low plasma sodium concentration (125 mmol/l) and the possible significance of this finding will be discussed below.

Discussion

The correlations illustrated in Figure 2 and listed above have two-fold significance:

1. *Factors affecting the* V_d *of digoxin*

It might reasonably be expected that the V_d of digoxin would, at least in part, be related to its binding to tissue sodium- and potassium- linked magnesium-dependent adenosine triphosphatase (Na^+-K^+-ATPase) as well as to its distribution within other cellular tissue components. These data suggest that the V_d of digoxin can be related both to the absolute amount of Na^+-K^+-ATPase activity in patients' tissues and to the sensitivity of that activity to inhibition by digoxin.

That the V_d may be related to the activity of Na^+-K^+-ATPase is suggested by the correlation between V_d and age (Figure 2b). Na^+-K^+-ATPase activity in human red cells has been shown to decrease with age (Platt & Schoch, 1974) so that if less Na^+-K^+-ATPase were available for binding digoxin the V_d might be expected to be diminished. This correlation also agrees with the data of Ewy, Kapadia, Yao, Lullin & Marcus (1969) who found that older patients had higher plasma concentrations of digoxin than younger patients following similar doses of drug and the difference may be interpreted as suggesting a lowered V_d in the older patients.

That the V_d is related to the sensitivity of red cell Na^+-K^+-ATPase to inhibition by digoxin is suggested by the correlation between V_d and IC_{50} (Figure 2a). The binding of digoxin to red cell membranes has been shown to be correlated with the consequent decrease in potassium influx into the cells (Gardner, Kiino, Swartz & Butler, 1973) and there is some evidence that the inhibition of potassium (or rubidium) influx is directly related to inhibition of Na^+-K^+-ATPase (for a review see Skou, 1965). In addition about 85% of rubidium influx into red cells can be inhibited by cardiac glycosides (Figure 1, inset). Thus the major part of rubidium influx seems to be linked to Na^+-K^+-ATPase activity which can be inhibited by cardiac

Table 1 Clinical and pharmacokinetic data of the 18 subjects

Patient number†	*Age (years)*	*Weight (kg)*	*IC_{50} (ng/ml)*	*C_{SS} (ng/ml)*	*V_d (l/kg)*
1	80	51.4	24.3	1.3	6.26
2	51	72.7	–	2.1	8.22
3	87	50.0	16.8	2.5	2.33
4	63	50.8	15.7	1.2	6.86
6	59	69.8	–	1.6	5.07
9	67	70.0	12.5	1.6	7.52
10	35	65.0	8.7	1.1	11.81
11	50	61.2	5.2	0.9	10.81
12	60	80.0	9.5	0.9	8.36
13	42	73.4	25.0	1.1	5.18
14	73	67.7	–	1.8	3.39
15	50	65.9	23.3	1.4	4.53
Patient A	61	59.3	7.3	0.7	7.33*
Patient B	58	79.8	5.8	1.3	9.68
Patient C	78	56.1	22.3	1.4	3.79
Volunteer 1	30	70.0	10.0	1.1	10.95
Volunteer 2	37	70.0	10.0	0.6	9.96
Volunteer 3	27	70.1	12.6	1.1	11.15

* V_d for this patient was actually measured using a two-compartment open model analysis following an intravenous dose of 0.5 mg and plasma concentration measurement up to 19 h. The value found was 7.96 l/kg.
† The numbering of patients corresponds with that in Table 1 of Aronson *et al.* (1977), p. 213.

glycosides. The link between binding to and inhibition of $Na^+-K^+-ATPase$ is yet to be substantiated.

The independence of the negative correlations between V_d and (a) IC_{50} and (b) age can be judged by the fact that the correlation between age and IC_{50} is not significant while the partial correlation coefficients for IC_{50} and age in the multiple regression are highly significant. Thus the two significant negative correlations are not spuriously caused by the dependent variable being by chance related to two variables which already correlate with each other. One would not in any case necessarily expect IC_{50} and age to correlate significantly with one another. Platt & Schoch (1974) demonstrated that the IC_{50} of the red cell $Na^+-K^+-ATPase$ of elderly patients was higher than that of young patients but they did not report sufficient data to be able to deduce a linear relationship between age and IC_{50}. Total $Na^+-K^+-ATPase$ activity will not necessarily correlate with the ease of inhibition of that activity when the inhibition is measured in the presence of other variables likely to alter the nature of the binding of digoxin to the enzyme (e.g. intra- and extracellular cationic concentrations). Indeed it has been shown (Welt, Smith, Dunn, Czerwenski, Proctor, Cole, Balfe & Gitelman, 1967) that the red cells of different patients may contain different activities of $Na^+-K^+-ATPase$ while the IC_{50} values of those activities are the same.

In the context of these theories on the determinants of V_d the one patient whose V_d deviates significantly from the rest in respect of IC_{50} (but not apparently of age, Figures 2a and 2b) raises an interesting point. Some patients with renal failure may have lowered V_d of digoxin (Koup, Jusko, Elwood & Kohli, 1975) and we have suggested elsewhere (Aronson & Grahame-Smith, 1976) that this may be due to lowered activity of ATPase. This hypothesis is based on the findings of Welt, Cole and their associates (Welt *et al.*, 1967; Cole, Balfe & Welt, 1968; Cole, 1973) who have shown that $Na^+-K^+-ATPase$ activity in the red cells of patients in renal failure, particularly those with high intraerythrocytic sodium concentrations, is lower than in the red cells of control patients (Cole, 1973), that plasma from uraemic patients with elevated intraerythrocytic sodium concentrations inhibits $Na^+-K^+-ATPase$ activity in normal erythrocytes (Cole *et al.*, 1968) and that the residual $Na^+-K^+-ATPase$ activity in inhibited red cells is as sensitive to the effects of ouabain as the ATPase in normal cells (Welt *et al.*, 1967). They have also suggested (Welt *et al.*, 1967) that these abnormalities are not confined to patients with renal failure but may occur in any very ill patient with high intraerythrocytic sodium concentrations. This has come to be known as the 'sick-cell syndrome'. The patient whose V_d differs significantly from the rest (Figures 2a and 2b) was one who had been treated with digoxin for atrial fibrillation due to chronic ischaemic heart disease but who had not taken her treatment for several weeks. She was admitted unconscious with a right hemiplegia and when she recovered consciousness digoxin therapy was restarted. Her condition, however, deteriorated over the next 2 weeks and she died. Her plasma sodium concentration during this time was abnormally low (mean 125 mmol/l) and it is conceivable that this was due to inhibition of her tissue $Na^+-K^+-ATPase$ in the manner described above. If that were so her IC_{50} would not differ from the value which would have been found in the absence of such an abnormality but, because of the diminished total activity of $Na^+-K^+-ATPase$, the V_d would be reduced (in this case from about 6.3 to 2.3 l/kg). The value of 6.3 l/kg in an 87 year old subject does not differ significantly from the other values shown in Figure 2b and is thus within the limits of V_d which one might expect in a subject of this age.

2. *Can digoxin requirements be predicted?*

There exists the possibility that, using the multivariate correlation equating V_d with both IC_{50} and age, the loading dose of digoxin which would result in a chosen plasma concentration would be predictable before therapy commenced, in patients with normal thyroid function, thus:

C_{SS} = loading dose/V_d (Wagner, Weidler & Lin, 1976)

If this were possible then more precise control of digoxin therapy would be attainable from the start of treatment resulting in a reduction in morbidity and mortality from digoxin toxicity.

Conclusion

It should be pointed out that the calculations of V_d in these studies are based on two assumptions (see section on pharmacokinetic methods), namely that the percentage absorption of drug is the same in all patients and that the $T_{\frac{1}{2}\beta}$ can be directly calculated from the ratio of loading dose to maintenance dose. The use of high bioavailability tablets increases the likelihood that percentage absorption in different patients is similar (Shaw, Raymond & Greenwood, 1975) and at values of around 67% absorption (Doherty & Kane, 1975) small differences will not greatly alter the final value of V_d. The method of calculation of β is more difficult to justify but it is encouraging that in patient A (Table 1) for whom V_d was directly measured the value obtained corresponds well with

the value found using the other method. Moreover if we ignore the contribution of F and β in the equation of Wagner *et al.* (1965) and merely calculate the ratio $D/C_{SS}.\tau$ the following correlations are found:

$D/C_{SS}.\tau$ *v.* IC_{50}, $r = -0.5391$, $P < 0.05$
$D/C_{SS}.\tau$ *v.* age, $r = -0.7383$, $P < 0.001$

Thus both IC_{50} and the patients' age correlate with the steady state plasma concentration resulting from a given daily maintenance dose the correlation in the case of age being no different to that between V_d and age. The better correlation of IC_{50} with the calculated V_d requires to be substantiated by more rigorous prospective studies.

References

ARONSON, J.K. & GRAHAME-SMITH, D.G. (1976). Altered distribution of digoxin in renal failure–a cause of digoxin toxicity? *Br. J. clin. Pharmac.*, 3, 1045-1051.

ARONSON, J.K., GRAHAME-SMITH, D.G., HALLIS, K.F., HIBBLE, A. & WIGLEY, F. (1977). Monitoring digoxin therapy: I. Plasma concentrations and an *in vitro* assay of tissue response. *Br. J. clin. Pharmac.*, 4, 213-221.

COLE, C.H. (1973). Decreased ouabain-sensitive adenosine triphosphatase activity in the erythrocyte membrane of patients with chronic renal disease. *Clin. Sci. Mol. Med.*, 45, 775-784.

COLE, C.H., BALFE, J.W. & WELT, L.G. (1968). Induction of an ouabain sensitive ATPase defect by uremic plasma. *Trans. Ass. Am. Phys.*, 81, 213-220.

DOHERTY, J.E. & KANE, J.J. (1975). Clinical pharmacology of digitalis glycosides. In *Annual Review of Medicine*, 26, pp. 159-171, eds, W.P. Creger, C.H. Coggins & E.W. Hancock. Annual Reviews Inc.

EWY, G.A., KAPADIA, G.G., YAO, L., LULLIN, M. & MARCUS, F.I. (1969). Digoxin metabolism in the elderly. *Circulation*, 39, 449-453.

GARDNER, J.D., KIINO, D.R., SWARTZ, T.J. & BUTLER, V.P. (1973). Effects of digoxin-specific antibodies on accumulation and binding of digoxin by human erythrocytes. *J. clin. Invest.*, 52, 1820-1833.

JOHNSON, B.F., BYE, C. & LADER, S. (1974). The bioavailability of other digoxin preparations compared with tablets. *Postgrad. Med. J.*, 50, (Suppl. 6), 62-66.

KOUP, J.R., JUSKO, W.J., ELWOOD, C.M. & KOHLI, R.K. (1975). Digoxin pharmacokinetics: Role of renal failure in dosage regimen design. *Clin. Pharmac. Ther.*, 18, 9-21.

PLATT, D. & SCHOCH, P. (1974). Effect of age and cardiac glycosides on the activity of adenosine triphosphatase (ATPase) (EC 3.6.1.3) of red cell ghost membranes. *Mechanisms of Ageing and Development*, 3, 245-252.

SHAW, T.R.D., RAYMOND, K. & GREENWOOD, H. (1975). The biological availability of very rapidly dissolving digoxin tablets. *Postgrad. med. J.*, 50 (Suppl. 6), 55-61.

SKOU, J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membrane. *Physiol. Rev.*, 45, 596-617.

SMITH, T.W., BUTLER, V.P. & HABER, E. (1969). Determination of therapeutic and toxic serum digoxin concentrations by radioimmunoassay. *New Eng. J. Med.*, 281, 1212-1216.

SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical Methods*, 6th Edition. Iowa: Iowa State University Press.

WAGNER, J.G., NORTHAM, J.I., ALWAY, C.D. & CARPENTER, O.S. (1965). Blood levels of drug at the equilibrium state after multiple dosing. *Nature, Lond.*, 207, 1301-1302.

WAGNER, J.G., WEIDLER, D.J. & LIN, Y.-J. (1976). New method for detecting and quantitating pharmacokinetic drug-drug interactions applied to ethanol-propranolol. *Res. Comm. chem. Path. Pharmac.*, 13, 9-18.

WELT, L.G., SMITH, E.K.M., DUNN, M.J., CZERWENSKI, A., PROCTOR, H., COLE, C.H., BALFE, J.W. & GITELMAN, H.J. (1967). Membrane transport defect: the sick cell. *Trans. Ass. Am. Phys.*, 80, 217-226.

(Received June 25, 1976)