THE ERYTHROCYTE SODIUM AND POTASSIUM IN PATIENTS TREATED WITH DIGOXIN

D.B. MORGAN, M. CUMBERBATCH, S. COHN, D. SCOTT,
T. GUNASUNTHARAM, C. DAVIDSON & C. CHAPMAN

Departments of Chemical Pathology, Nuclear Medicine,
The General Infirmary, Leeds and Birch Hill Hospital, Rochdale

- 1 Four healthy persons and ten patients with heart failure were studied for 5 to 20 days after they started taking digoxin. The sodium content of their erythrocytes increased and there was an equimolar decrease in potassium content.
- 2 The increase in erythrocyte sodium for a given increase in plasma digoxin during this acute digitalization was less on average and varied more in the patients than in the healthy persons, that is the patients' erythrocytes were less responsive to digoxin.
- 3 The average erythrocyte sodium was greater in 183 patients who had been taking digoxin for at least 2 months than in 100 healthy persons not taking digoxin but there was no significant correlation between the plasma digoxin concentrations and erythrocyte sodium concentration in the patients. Indeed, there was no apparent change in the erythrocyte sodium in many of the patients taking digoxin.
- 4 If the erythrocyte sodium concentration is a reliable guide to the tissue effects of digoxin then the results suggest that there is a wide variation in the response to digoxin between patients both during acute digitalization and during chronic treatment with digoxin.

Introduction

The therapeutic value of digoxin is undoubted but digoxin toxicity remains a problem in clinical practice. The reasons are that there is little difference between the useful and the toxic dose; the symptoms and arrhythmias which digoxin can cause are not specific and the onset of toxicity is unpredictable in relation to the dose and to the duration of digoxin treatment (Chung, 1969). It is likely that because of this unpredictability many patients treated with digoxin (perhaps 20%) are given less than the maximally effective dose (Smith, 1975).

Theoretically the difficulty in predicting digoxin toxicity could be due to variation between patients in the blood levels of digoxin after a particular dose or to variation in the organ responses to a particular blood level of digoxin. Plasma digoxin measurements, although heralded as a great advance, do not by any means completely distinguish patients with 'toxic' effects from those without, presumably because of a variation in the organ responsiveness to digoxin (Smith, 1975; Aronson, Grahame-Smith & Wigley, 1978). It has even been suggested that there is no study which clearly demonstrates a role for plasma digoxin measurements in the detection or exclusion of digoxin 'toxicity' (Ingelfinger & Goldman, 1976; Lasagna, 1976).

The ideal approach to the detection of digoxin

toxicity would be to measure a cell effect of digoxin. Digoxin like other cardiac glycosides can inhibit the Na-K-ATPase in the erythrocyte (Glynn, 1964) and cardiac muscle (Gibson & Harris, 1968). This inhibition has been regarded as the explanation for the therapeutically useful inotropic effect of digoxin (Aker & Brody, 1978), but some authors suggest that it is only responsible for the 'toxic' effects of digoxin, in particular, the dysrhythmias (Okita, Teneick & Richardson, 1974).

Little Na-K-ATPase activity can be measured in erythrocytes *in vitro* unless the erythrocytes are broken (to give the added ATP access to the inner surface of the cell membrane), and their membranes are fragmented (Post, Merritt, Kinsolving & Albright, 1960). Digoxin added *in vitro* to these membrane preparations inhibits Na-K-ATPase activity, and this effect has been taken as the basis of a digoxin assay (Burnett & Conklin, 1971).

An easily detected effect of the inhibition of Na-K-ATPase *in vitro* is an increase in the erythrocyte content of sodium which is associated with a decrease in the erythrocyte content of potassium (Gill & Solomon, 1959).

Several authors have detected these changes in erythrocyte sodium and potassium in patients taking digoxin and Wessels, Samizadeh & Losse (1974) and

Loes, Singh, Lock & Mirkin (1978) suggested that extreme changes could be used to detect patients with digoxin toxicity.

We have examined the changes in erythrocyte sodium and potassium and their relation to plasma digoxin in a longitudinal study in four healthy persons and ten patients with heart failure during acute digitalization, and in a cross-sectional study of 183 patients who had been taking digoxin regularly for at least two months.

Methods

Four healthy persons and ten patients admitted to hospital with congestive heart failure were each digitalized with digoxin over a period of days. The dose was 0.25 mg a day in the healthy persons, whereas the patients were given either 0.5 mg twice on the first day and then 0.25 mg twice a day or 0.75 mg a day for 2 days, 0.5 mg a day for 4 days and then 0.25 mg a day. Blood samples were taken every 1 or 2 days for 5 to 20 days from the beginning of digitalization.

In the cross-sectional study, there were 183 patients who had been taking digoxin for at least 2 months and in the same dose for at least 1 month. They were attending the routine cardiology clinics or had been admitted to hospital from the waiting list for further assessment. They were all taking diuretics and potassium supplements and none of them had a plasma potassium less than 3.0 mmol l^{-1} . None of these patients had the symptoms or signs of digoxin toxicity. Blood samples were taken from these patients before their morning dose of digoxin.

Erythrocyte sodium and potassium content was also measured in a group of 100 control subjects who were either blood donors, medical students or laboratory staff.

Heparinized blood was centrifuged at $2500 g$ for 10 min and the plasma sodium and potassium were measured by flame photometry using standard AutoAnalyser techniques. The packed cells were centrifuged at $15,000 g$ for 30 min in soft polythene tubes (500 μl) in a Beckman Microfuge. Each tube was cut 3 and 20 mm from the tip, and the middle segment was weighed and dropped into 10 ml of lithium sulphate (15 mmol l^{-1}). The solution was shaken vigorously to remove all the packed cells from the segment and lyse them. The empty segment of tube was removed from the haemolysate, dried and weighed and the weight of packed cells which had been haemolysed was calculated by difference. The sodium and potassium concentrations in the haemolysate were measured by flame photometry. Two samples of packed cells were centrifuged and lysed from each blood sample and the values given in the text are based on the mean values obtained for

Figure 1 The sodium ($[\text{Na}^+]_{\text{RBC}}^{\text{uw}}$, ●) and potassium ($[\text{K}^+]_{\text{RBC}}^{\text{uw}}$, ○) content of unwashed erythrocytes (mmol kg^{-1}) and the plasma digoxin (ng ml^{-1}) before, during and after the indicated daily dose of digoxin in a patient who required digitalization

these two samples. No correction was made for the effect of the trapped plasma on the sodium concentration of the packed red cells, but the volume of trapped plasma was constant ($3.3 \pm 0.1\%$) and differences in the given values indicate differences in true erythrocyte sodium and potassium.

Plasma digoxin was measured by radioimmunoassay using a kit with I^{125} -tyrosyl-digoxin (The Radiochemical Centre, Amersham, Bucks).

Results

In the acute studies, there was a rise in plasma digoxin to peak values of between 1 and 2 ng ml^{-1} in the healthy persons and between 1 and 5 ng ml^{-1} in the patients, and this rise was followed by a fall when the digoxin was stopped or the dose was reduced. This rise and fall in plasma digoxin was associated with a rise and fall in erythrocyte sodium and a fall and rise in erythrocyte potassium (Figure 1). Table 1 shows the erythrocyte sodium and potassium at the peak plasma digoxin in the healthy persons and patients. The peak erythrocyte sodium and potassium were similar in the two groups although the plasma digoxin was two-and-a-half times higher in the patient group. Figure 2a shows the relationship between erythrocyte sodium and plasma digoxin in

Figure 2 The relation between the sodium content of unwashed erythrocytes ($[Na^+]_{RBC}^{uw}$ mmol kg^{-1}) and the plasma digoxin ($ng\ ml^{-1}$) during acute digitalization. Each line is the calculated linear regression of erythrocyte sodium on plasma digoxin calculated by the method of least squares, from at least five pairs of values; the coefficients of linear correlation never being less than 0.7.

- a) The relation in four control subjects (continuous lines), the interrupted lines are the predicted limits of the relationship calculated from the extreme values of erythrocyte sodium content in healthy subjects, and the extreme slopes observed in the four control subjects.
- b) The relation in ten patients, with heart failure, during digitalization (continuous lines). The interrupted lines are those shown in (a).

each of the four healthy persons during the rise in plasma digoxin, and also shows the limits of the relationship as calculated from the range of erythrocyte sodium in healthy persons and the observed range of the increase in erythrocyte sodium per unit change in plasma digoxin. Figure 2b shows the relationship between erythrocyte sodium and plasma digoxin during digitalization in the 10 patients. There was considerable variability between patients in the slope of the relationship but in most of the patients the slope was less than that observed in

the health controls. The mean of the slope of the regression line was $2.39\ mmol\ ng^{-1}$ (range 0.54 to 4.95) in the patients and $5.86\ mmol\ ng^{-1}$ (range 5.11 to 6.92) in the controls.

Table 1 shows the erythrocyte sodium and potassium in the 183 patients on chronic digoxin treatment and in 100 untreated healthy controls. There was only a small increase in the average erythrocyte sodium, which failed to show any clear correlation with the plasma digoxin (Figure 3), and no decrease in the average erythrocyte potassium

Table 1 Descriptive statistics (mean and s.e. mean) of erythrocyte sodium and potassium ($mmol\ kg^{-1}$) and plasma digoxin ($ng\ ml^{-1}$) in healthy controls and subjects taking digoxin

Group	n	Plasma digoxin ($ng\ ml^{-1}$)	Erythrocyte ($mmol\ kg^{-1}$) Sodium	Potassium
Controls	100	—	10.3 (0.14)	86.7 (0.59)
Acute				
Healthy volunteers	4	1.1 (0.12)	15.5* (1.46)	82.3† (2.24)
Patients with heart failure	10	2.6 (0.48)	15.9* (1.59)	81.2† (1.51)
Chronic				
Patients with heart failure	183	1.8 (0.05–5.0)	11.6* (0.20)	87.7† (0.39)

Differences between group means were assessed by the two tailed Student's *t*-test

* $P < 0.001$, † $P < 0.005$, ‡ $P < 0.2 > 0.1$

Figure 3 The relation between the sodium content of unwashed erythrocytes ($[Na^+]_{RBC}^{uw}$ mmol kg⁻¹) and the plasma digoxin (ng ml⁻¹) in single samples from 183 patients who had been taking digoxin for at least 2 months.

although the mean plasma digoxin was greater than in the acute studies in the controls.

Figure 4 shows that during acute digitalization the changes in erythrocyte sodium and potassium were equimolar. In the chronically treated group only the observed erythrocyte sodium and potassium can be compared but they are related (Figure 5), the slope of the relationship being different from zero but not very different from 1 ($r = -0.365$, $n = 183$, $P < 0.001$, slope = -0.72 95% confidence interval -0.45 to -0.99).

Discussion

The inhibitory effect of cardiac glycosides on the Na-K-ATPase of erythrocytes is well known but there have been few studies which demonstrate the expected increase in erythrocyte sodium and decrease in erythrocyte potassium in patients taking digoxin (Clifford & Bentyman, 1958; Kettlewell, Nowers & White, 1971; Astrup, 1974; Wessels *et al.*, 1974; Wessels, Samizadeh, Heinze & Tasche, 1975; Funder & Weith, 1975; Burck *et al.*, 1975; Bahemuka & Hodgkinson, 1976; Loes *et al.*, 1978). Four of these were studies of acute digitalization (Table 2) but only three of them included measurements of erythrocyte

Figure 4 The relation between the change in potassium content ($[K^+]_{RBC}^{uw}$) and sodium content ($[Na^+]_{RBC}^{uw}$) of unwashed erythrocytes (mmol kg⁻¹) during acute digitalization of four control subjects and ten patients with heart failure ($r = -0.87$, $n = 111$, $P < 0.001$, slope = -1.19 95% confidence interval -1.12 to -1.27).

sodium. The changes in erythrocyte sodium in our patients during acute digitalization were greater than in the previous studies but this could be due to differences in the plasma digoxin which are only given for Loes *et al.* (1978).

Four of the previous studies were in patients who had been taking digoxin for some time (Table 2), Funder & Weith (1974) detected a rise in the erythrocyte sodium in their patients but little fall in the erythrocyte potassium. Wessels *et al.* (1974) and Loes *et al.* (1978) detected a fall in erythrocyte potassium as well as a rise in erythrocyte sodium in their patients, especially those with the symptoms and signs of digoxin toxicity in whom the changes in erythrocyte sodium were much greater than those reported in any other study (Table 2). Bakemuka & Hodgkinson (1976) reported a large decrease in erythrocyte potassium in their elderly patients, most of whom were convalescent after a hospital admission for heart failure. Our patients on long term digoxin therapy had a raised mean erythrocyte sodium but no significant reduction in mean erythrocyte potassium. On the other hand there was a clear-cut fall in the erythrocyte potassium in our patients during acute digitalization and a negative relationship between erythrocyte sodium and potassium in the 183 patients on long term digoxin therapy. These results suggest that erythrocyte potassium may not be a good guide to the effect of digoxin during long term treatment, there are probably two reasons for this. The first is

Table 2 The effect of digoxin on erythrocyte sodium and potassium

Series	Type ^a	Duration	Sodium (mmol kg ⁻¹)			Potassium (mmol kg ⁻¹)			Δ Na - Δ K
			Controls n	mean	Δ D-C	Controls n	mean	Δ D-C	
Clifford & Bentyman (1958)	A	3-10 days	—	—	—	10	92.3	85.3	-7.0
Kettlewell <i>et al.</i> (1971)	A	48h	12	11.2	+1.2	12	92.0	87.3	-4.7
Astrup (1974)	A	10 days	21	10.4	+4.4	21	90.5	86.6	-3.9
Loes <i>et al.</i> (1978)	A	24h	11	9.6	+5.2	11	95.8	90.5	-5.4
Present study	A	12 days 5-20 days	100	10.3	+5.1	100	86.7	82.3	-4.4
			10 ^f	15.9	+5.6	10	81.2	81.2	-5.5
Funder & Weith (1974)	C	5.5	128	11.2	+7.8	128	91.5	89.4	-2.1
Wessels <i>et al.</i> (1974)	C	> 14 days	31	9.9	+0.6	31	88.9	82.0	-6.9
			13 ^b	22.8	+12.9	13	71.0	71.0	-17.9
Bahemuka & Hodgkinson (1976)	C	S.S.	—	—	—	140	90.6	78.1	-12.5
Loes <i>et al.</i> (1976)	C	S.S.	11	9.6	+2.3	11	95.8	92.5	-3.3
			11 ^b	21.2	+11.5	11	86.7	87.7	+1.0
Present study	C	S.S.	100	10.3	+1.2	100	86.7	87.7	+1.0

t: A, longitudinal; C, cross sectional; S.S.: Steady state long term treatment; a, not toxic; b, obvious digoxin toxicity; v, healthy volunteers; f, patients with heart failure.

Figure 5 The relation between the potassium ($[K^+]_{RBC}^{uw}$) and sodium ($[Na^+]_{RBC}^{uw}$) content of unwashed erythrocytes (mmol kg^{-1}) in single samples from 183 patients who had been taking digoxin for at least 2 months ($r = -0.365$, $n = 183$, $P < 0.001$, slope $= -0.72$ 95% confidence interval -0.45 to -0.99).

that the average effect of digoxin, as indicated by the increase in the erythrocyte sodium, was much less in the cross-sectional study than in the acute study. The second is that although the fall in erythrocyte potassium is of the same magnitude in molar terms as the increase in erythrocyte sodium it is a much smaller proportional change, and may therefore be statistically insignificant when the change in erythrocyte sodium is highly significant.

Although the measurement of erythrocyte potassium may not be a sensitive guide to the effects of digoxin during long term treatment, all the studies, short term and long term, show that digoxin lowers the erythrocyte potassium as well as increasing the erythrocyte sodium (Table 2). In four of the five short

term studies the ratio between the increase in erythrocyte sodium and fall in erythrocyte potassium was close to 1 (Table 2) it was 0.26 in the other. In the previous long term studies the ratio of the changes ranged from 0.1 to 3.7.

Plasma digoxin is known to be a poor guide to the cellular effects of digoxin. On the other hand, the erythrocyte sodium is determined by the inhibitory effect of digoxin on the Na-K-ATPase activity and might therefore be a better guide to the effects of digoxin, as suggested by Wessels *et al.* (1974, 1975) and Grahame-Smith (1978).

There have been no previous studies of the relationship between the changes in erythrocyte sodium and plasma digoxin. Our studies show that there is such a relationship during acute digitalization in health persons and in patients. However, the effect of a given plasma digoxin was more variable and on average less in the patients than in the healthy controls. The increase in erythrocyte sodium was even less in the patients chronically treated with digoxin and in some of them there was no apparent increase in the erythrocyte sodium even when the plasma digoxin was as high as 1.5 ng ml^{-1} .

A similar reduction in the effect of digoxin on erythrocyte sodium and potassium transport during long term treatment has been suggested recently by Grahame-Smith (1978) who measured ^{86}Rb uptake into erythrocytes as an index of potassium and therefore sodium transport. This reduced effect was associated with an increase in the number of binding sites for ouabain and therefore presumably with an increase in the amount of Na-K-ATPase in the membrane.

If the effect of digoxin on sodium transport in the erythrocyte is an index of its inotropic effect on the myocardium, then our results indicate a wide variation in this effect during short- and long-term treatment with digoxin and suggest a simple technique by which this effect can be assessed and monitored in the individual. There is an increased need for such a technique when the case for continuing digoxin in patients with sinus rhythm is being questioned (Guz & McHaffie, 1978; Johnston & McDevitt, 1979).

References

- AKERA, T. & BRODY, T.M. (1978). The role of Na^+ , K^+ -ATPase in the inotropic action of digitalis. *Pharmac. Rev.*, **29**, 187–220.
- ARONSON, J.K., GRAHAME-SMITH, D.G. & WIGLEY, F.M. (1978). Monitoring digoxin therapy: The use of plasma digoxin concentration measurements in the diagnosis of digoxin toxicity. *Quart. J. Med.*, **186**, 11–22.
- ASTRUP, J. (1974). The effect of hypokalaemia and of digoxin therapy on red cell sodium and potassium content; some clinical aspects. *Scand. J. clin. lab. Invest.*, **33**, 11–16.
- BAHEMUKA, M. & HODKINSON, H.M. (1976). Red blood cell potassium as a practical index of potassium status in elderly patients. *Age & Ageing*, **5**, 24–30.
- BURK, H.C., HAASIS, R. & LARBIG, D. (1975). Beeinflussung der erythrocyte-elektrolyte durch β -methyl digoxin bei gesunden. *Klin. Wschr.*, **53**, 125–128.
- BURNETT, G.H. & CONKLIN, R.L. (1971). The enzymatic

- assay of plasma digoxin. *J. lab. clin. Med.*, **78**, 779–783.
- CHUNG, E.K. (1969) Digoxin. Excerpta Medica Foundation, Amsterdam.
- CLIFFORD, T.C. & BENTYMAN, W. (1958). Changes in erythrocyte potassium in patients with cardiac failure treated with digitalis. *Clin. Chem.*, **4**, 311–315.
- FUNDER, J. & WEITH, J.O. (1974). Combined effects of digitalis therapy and of plasma bicarbonate on human red cell sodium and potassium. *Scand. J. clin. Invest.*, **34**, 153–160.
- GIBSON, K. & HARRIS, P. (1968). Some properties of human myocardial microsomal sodium-potassium ATPase. *Cardiovascular Res.*, **2**, 367–370.
- GILL, T.J. & SOLOMON, A.K. (1959). Effect of ouabain on sodium flux in human red cells. *Nature*, **183**, 1127–1128.
- GLYNN, I.M. (1964). The action of cardiac glycosides on ion movements. *Pharmac. Rev.*, **16**, 381–407.
- GRAHAME-SMITH, D.G. (1978). The Foxglove 1978: Some aspects of the clinical pharmacology of digoxin. Part V In *Developments in cardiovascular medicine*, eds. Dickinson, C.J. & Mortis, J. Lancaster: MTP Press Ltd.
- GUZ, A. & McHAFFIE, D. (1978). The use of digitalis glycosides in sinus rhythm. *Clin. Sci. mol. Med.*, **55**, 417–421.
- INGELFINGER, J.A. & GOLDMAN, P. (1976). The serum digitalis concentration—Does it diagnose toxicity? *New Engl. J. Med.*, **294**, 867–870.
- JOHNSTON, G.D. McDEVITT, D.G. (1979). Is maintenance digoxin in patients with sinus rhythm. *Lancet*, **i**, 567–570.
- KETTLEWELL, M., NOWERS, A. & WHITE, R. (1971). Effect of digoxin on human red blood cell electrolytes. *Br. J. Pharmac.*, **44**, 165–167.
- LASAGNA, L. (1976). How useful are serum digitalis measurements? *New Engl. J. Med.*, **294**, 898–899.
- LOES, M.W., SINGH, S., LOCK, J.E. & MIRKIN, B.L. (1978). Relation between plasma and red-cell electrolyte concentrations and digoxin levels in children. *New Engl. J. Med.*, **299**, 501–504.
- OKITA, G.T., TENEICK, R.E. & RICHARDSON, F. (1974). Inhibition of Na⁺, K⁺-ATPase and digitalis action: dissociation from ionotropic effects and its role in digitalis cardiotoxicity. *Ann. N.Y. Acad. Sci.*, **264**, 658–669.
- POST, R.L., MERRITT, C.R., KINSOLVING, C.R. & ALBRIGHT, C.D. (1960). Membrane ATPase as a participant in the active transport of sodium and potassium in the human erythrocyte. *J. biol. Chem.*, **255**, 1799–1802.
- SMITH, T.W. (1975). Digitalis toxicity: Epidemiology and clinical use of serum concentration measurements. *Am. J. Med.*, **58**, 470–476.
- WESSELS, F., SAMIZADEH, A. & LOSSE, H. (1974). Diagnosis der digitalisintoxikation durch bestimmung der erythrocyten-elektrolyte. *Klin. Wschr.*, **52**, 341–453.
- WESSELS, F., SAMIZADEH, A., HEINZE, A. & TASCHE, V. (1975). Erythrozytenelektrolyte als parameter einer wirksamen digitalisierung. *Verh. dt. Ges. inn Med.*, **81**, 1672–1674.

(Received October 19, 1979)