In vivo cation transport during short-term and long-term digoxin therapy

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1 We have studied the effects of digoxin on cation transport *in vivo* by measuring the changes in plasma and red cell rubidium concentrations following an oral load of rubidium chloride.

2 In eight patients who had been taking digoxin for 7 to 10 days (mean plasma digoxin concentration 1.3 ng ml^{-1}) the rise in plasma rubidium concentrations was enhanced and the rise in red cell rubidium concentrations was attenuated following the oral load of rubidium chloride, by comparison with the changes in well-matched controls.

3 In contrast, the disposition of rubidium was not altered in 12 patients who had been taking digoxin for more than 3 months (mean plasma digoxin concentration 1.1 ng ml^{-1}).

4 These results suggest that the inhibitory effects of digoxin on cation transport are detectable *in vivo* during short-term therapy, but not during long-term therapy, and confirm our previous *in vitro* findings.

Keywords digoxin cation transport pharmacological tolerance

Introduction

Digoxin is a potent and specific inhibitor of Na^+ , K^+ -ATPase, and this may be the mechanism by which it exerts some of its therapeutic effects (for reviews see Schwartz *et al.*, 1975; Noble, 1980). *In vitro* studies on the erythrocytes of patients during short-term digoxin treatment have shown changes that are consistent with occupation of Na^+ , K^+ -ATPase sites and a reduction in Na^+ , K^+ -ATPase activity—for example, specific [^3H]-digoxin binding, ouabain-sensitive $^{86}\text{rubidium}$ uptake, and ouabain-sensitive sodium efflux are reduced, and intra-erythrocytic sodium concentrations are increased (Ford *et al.*, 1979a; Zannad *et al.*, 1981; Cumberbatch *et al.*, 1981). However, changes of this kind cannot be detected in the erythrocytes of patients who have been taking digoxin for more than 2 months (Ford *et al.*, 1979b; Cumberbatch *et al.*, 1981). Thus, the inhibitory effect of digoxin on the Na^+ , K^+ -ATPase of erythrocytes may be subject to pharmacological tolerance, due to a compensa-

tory increase in the number of membrane-bound sodium pump sites, as measured *in vitro* (Ford *et al.*, 1979b).

Our purpose in this investigation was to study the effects of both short-term and long-term digoxin therapy using a technique we have developed to study cation transport *in vivo* rather than *in vitro*.

These data have been published in preliminary form elsewhere (Boon *et al.*, 1984a). The study had the approval of the Central Oxford Research Ethics Committee.

Methods

The disposition of rubidium

The details and the rationale of this method have already been published (Boon *et al.*, 1984b). In brief, each subject was given a total of 8 mg kg^{-1} of rubidium chloride, divided into eight equal

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aliquots given orally at 15 min intervals. Venous blood samples were taken before the first dose, and 1 h and 5 h after the last dose of rubidium chloride. Plasma and intra-erythrocytic rubidium concentrations were measured by graphite furnace atomic absorption spectrophotometry (Hallis *et al.*, 1985), as were plasma and intra-erythrocytic potassium concentrations. Plasma digoxin concentrations were measured by radioimmunoassay.

Subjects

Study 1: The effect of short-term digoxin therapy

The disposition of rubidium was studied in eight patients who had been taking digoxin for between 7 and 10 days. Each patient had taken a loading dose of between 0.5 and 1 mg, followed by a daily dose of 0.25 mg digoxin as treatment for heart failure or atrial fibrillation complicating ischaemic heart disease (6), recurrent supraventricular tachycardia (1), or heart failure due to a congestive cardiomyopathy (1). At the time of the study four patients were in sinus rhythm and four in atrial fibrillation. All but one of the patients were taking other drugs, including warfarin (4), frusemide (3), hydrochlorothiazide and amiloride (2), atenolol (1), glibenclamide (1), and allopurinol (1). The findings were compared with those in eight well-matched control subjects (for details see Table 1) using Student's unpaired *t*-test.

Study 2: The effect of long-term digoxin therapy

The disposition of rubidium was studied in 12 patients who had been taking digoxin for more than 3 months (median 18 months, range 3–144 months). Eleven patients were taking 0.25 mg and one 0.5 mg digoxin daily as treatment for heart failure complicating ischaemic heart disease (7), aortic valve disease (4), or mitral valve disease (1), and they were all in sinus rhythm. Nine of these patients were taking other drugs, including diuretics (8), atenolol (2), warfarin (2), and amiodarone (1). The findings were compared with those in 12 well-matched control

subjects (for details see Table 1) using Student's unpaired *t*-test.

Results

Study 1: The effect of short-term digoxin therapy

The mean plasma digoxin concentration immediately before the rubidium load was 1.3 ng ml⁻¹ (range 0.8–1.9 ng ml⁻¹). The patients receiving short-term digoxin therapy had a greater rise in plasma rubidium concentration and a smaller rise in intra-erythrocytic rubidium concentration, following the oral load of rubidium chloride, compared with the control subjects (Figure 1a).

Study 2: The effect of long-term digoxin therapy

The mean plasma digoxin concentration immediately before the rubidium load in the patients receiving long-term digoxin therapy was 1.1 ng ml⁻¹ (range 0.7–1.5 ng ml⁻¹). There were no significant differences in the disposition of rubidium between the patients receiving long-term digoxin therapy and their respective controls (Figure 1b).

Discussion

Transmembrane cation transport in man has generally been studied in isolated cells *in vitro*. However, *in vitro* findings may not always accurately reflect events *in vivo*. For example, techniques used in preparing cells from whole blood may alter cation transport directly (Poston *et al.*, 1982), or may result in the removal or inactivation of plasma-borne factors which influence cation transport *in vivo* (de Wardener & Clarkson, 1982). Furthermore, some *in vitro* techniques used for studying cation transport rely on artificial perturbations of intracellular and extracellular cation concentrations (Garay & Meyer, 1979), making it difficult to interpret any observed abnormalities.

We have therefore devised a simple method for studying cation transport *in vivo*, by measur-

Table 1 Details of the matching of patients on short-term and long-term digoxin therapy with their respective controls. Data are given as means (complete ranges)

	Controls	Short-term digoxin (7–10 days)	Controls	Long-term digoxin (> 3 months)
Number	8	8	12	12
Men	5	5	10	10
Age (years)	62.2 (52–70)	64.6 (58–72)	62.0 (38–70)	60.0 (41–71)
Weight (kg)	65 (53–96)	69 (64–91)	73.0 (53–96)	73.0 (60–87)
Plasma K ⁺ (mmol l ⁻¹)	4.4 (4.0–4.9)	4.4 (3.2–5.7)	4.4 (4.1–4.7)	4.2 (4.1–4.6)
Red cell K ⁺ (mmol l ⁻¹)	83.6 (77–90)	81.2 (67–96)	85 (77–90)	88 (84–94)

Figure 1 The mean ($\pm$ s.d.) increases in plasma and intra-erythrocytic rubidium concentrations at 1 h and 5 h after an oral load of rubidium chloride in: (a) eight patients on short-term digoxin therapy (▨) and eight well-matched control subjects (□); and (b) twelve patients on long-term digoxin therapy (▤) and twelve well-matched control subjects (□)

ing the changes in plasma and intra-erythrocytic rubidium concentrations following the oral administration of rubidium chloride. The changes in plasma rubidium concentrations reflect the overall transport of rubidium out of the plasma into the intracellular compartment, whilst the changes in intra-erythrocytic rubidium concentrations reflect transport into a single cellular compartment, which may or may not be representative of the intracellular compartment as a whole.

We have previously shown that the disposition of rubidium is altered in healthy volunteers who have taken a loading dose of digoxin (Boon *et al.*, 1984b), and in this study we have found similar changes in patients who have been taking digoxin in maintenance dosage for less than 10 days. These alterations are in a direction which is

consistent with a generalized reduction in the activity of the Na^+, K^+ -ATPase. However, in patients who had been taking digoxin for more than 3 months there was no evidence of such inhibition, suggesting pharmacological tolerance to the short-term inhibitory effects of digoxin on the Na^+, K^+ -ATPase, and confirming the previous *in vitro* findings that we and others have described (Ford *et al.*, 1979b; Cumberbatch *et al.*, 1981).

These data therefore support the hypothesis that digoxin therapy produces an initial widespread reduction in cell membrane Na^+, K^+ -ATPase activity which does not persist during long-term therapy.

We gratefully acknowledge the support of the British Heart Foundation (NAB and JKA) and the Wellcome Trust (JKA).

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(Received 9 October 1985,
accepted 2 April 1986)